LABORATORY EXPERIMENTS IN DAIRY CHEMISTRY

BY

LEROY S. PALMER, PH.D.

PROFESSOR OF AGRICULTURAL BIOCHEMISTRY
UNIVERSITY OF MINNESOTA

NEW YORK
JOHN WILEY & SONS, INC.
LONDON: CHAPMAN & HALL, LIMITED
1926

THE WILEY AGRICULTURAL SERIES

EDITED BY

J. G. LIPMAN, B.Sc., A.M., PH.D., D.Sc.

DEAN OF AGRICULTURE, RUTGERS COLLEGE AND THE STATE UNIVERSITY OF NEW JERSEY

LABORATORY EXPERIMENTS IN DAIRY CHEMISTRY

THE WILEY AGRICULTURAL SERIES

EDITED BY

J. G. LIPMAN

Agricultural Marketing.

By JOHN T. HORNER, Professor in Economics, Michigan State College. viii + 249 pages. 6 by 9. 46 figures. Cloth, $2.50 *net.*

Beef Cattle.

Their Feeding and Management. By ROSCOE R. SNAPP, Assistant Professor of Animal Husbandry, University of Illinois. vi + 449 pages. 6 by 9. 85 figures. Cloth, $4.00 *net.*

Essentials of Systematic Pomology.

By BROOKS D. DRAIN, Assistant Professor of Pomology, Massachusetts Agricultural College. vi+284 pages. 6 by 9. 106 figures. Cloth $2.75 *net.*

Soil Management.

By FIRMAN E. BEAR, Professor of Soils, The Ohio State University; Associate in Soils, The Ohio Agricultural Experiment Station. vi + 268 pages. 6 by 9. 33 figures. Cloth, $2.00 *net.*

Principles of Dairying — Testing and Manufacture.

By H. F. JUDKINS, B.S., Head of Department of Dairying, Massachusetts Agricultural College, Amherst, Mass. xvii + 279 pages. 6 by 9. 74 figures. Cloth, $2.00 *net.*

Genetics in Plant and Animal Improvement.

By DONALD FORSHA JONES, Connecticut Agricultural Experiment Station, New Haven. v + 568 pages. 6 by 9. 229 figures. Cloth, $4.00 *net.*

Animal Breeding.

By LAURENCE M. WINTERS, M.S., Professor of Animal Husbandry, University of Saskatchewan. x + 309 pages. 6 by 9. 65 figures. Cloth, $2.75 *net.*

Printed in U. S. A.

Stanhope Press
TECHNICAL COMPOSITION COMPANY
F. H. GILSON COMPANY
BOSTON, U. S. A.

PREFACE

These exercises were prepared for the laboratory class in Course 103 of the Division of Agricultural Biochemistry, University of Minnesota. They supplement the lectures in Dairy Chemistry and are a part of the class work in that course. The exercises are largely biochemical in nature and are intended to give the student a familiarity with milk and its products from a biochemical point of view. Most of the exercises can be completed in thirty laboratory periods of two and a half hours each. The present outline is based on six years' experience at the University. The author can accordingly give reasonable assurance that the experiments will " work " in the hands of the student with average competence.

Most of the laboratory courses in Dairy Chemistry with which the author is familiar are confined largely to analysis and are in reality merely specialized courses in Quantitative Agricultural Analysis. There is very little quantitative analysis in this course. If desired, however, the course can be readily expanded to include the usual determinations. For example, in Chapter IV the physical and chemical constants of butterfat can be added. Directions for the determination of these constants are given in many texts. Similarly, Chapter VIII can be expanded to include tests for preservatives, nitrogen determinations, etc., directions for which are also readily accessible to the instructor. In the author's opinion, however, laboratory experiments of this nature fit more naturally into courses in Agricultural Analysis or Dairy Technology. This applies also to the analysis of milk as given in Chapter VIII. Most institutions now have the Mojonnier apparatus as part of their equipment. If expedient, the Mojonnier method can be

used to replace the directions given in Chapter VIII. Nevertheless, there is a certain cultural advantage to be gained in carrying out the analysis by the older method, even if it is to be replaced in practice by the more elaborate equipment.

The experiments outlined in this manual have been designed to coincide with the equipment of the Department with which the author is associated. Inasmuch as viscosimeters, tensiometers, hydrogen-ion apparatus, and ultramicroscopes are rapidly becoming necessary research equipment in all well-appointed laboratories, the author has ventured to make his undergraduate students familiar with them.

The chemistry training prerequisite for this course at the University of Minnesota is 10 quarter credits in General Chemistry and 10 quarter credits in General Agricultural Biochemistry which includes two quarters of General Agricultural Quantitative Analysis. In addition it is expected that the student has had a laboratory training such as usually accompanies an elementary course in dairying. It is the author's conviction that this is the minimum prerequisite training. It might well be augmented by a regular elementary course in organic chemistry, both lectures and laboratory. An advanced course in Dairy Products is not essential, but the Dairy Chemistry parallels or follows such a course splendidly.

The author is indebted to his laboratory assistants, Mr. G. A. Richardson, Mr. Otto Johnson, Mr. M. M. Miller, and Mr. Earl Jertsen, for assistance in formulating the procedure of many of the experiments in such a way that the chances for failure in the hands of the students are reduced to a minimum.

L. S. PALMER.

ST. PAUL, MINNESOTA
September 15, 1925

CONTENTS

GENERAL INSTRUCTIONS

The students are urged to read and study carefully the directions for each exercise before proceeding with the work. The more advanced student may secure a proper understanding of the significance of each exercise and its operation at the class period. The student less skilled in chemistry should make this preparation *before* the class period.

A considerable number of the exercises call for more or less definite quantities of milk and various reagents. Careful judgment is necessary as to the accuracy of measurement required, since this is not always indicated. The use of discernment in these cases is intended to be a part of the training given.

A list of desk equipment and general desk reagents is given for the convenience of the instructor. The equipment for each student requires a drawer and a locker as the minimum space. More space is desirable if available. One set of desk reagents can be used conveniently by two students working opposite each other. It will be found to be good training for the students to have them prepare the desk reagents at the opening class period when the desks are put in order and the equipment checked against the list furnished.

The list of special requirements for each experiment will be found to be a valuable aid to the instructor or his assistant. These can be kept on a special shelf or table. A number of the reagents can be used in a subsequent session if properly labeled and stored.

The type of note book or laboratory report required is left to the wishes of the instructor. Some type of report, however, is essential.

DESK REAGENTS

Conc. H_2SO_4	500 cc.
10 per cent HNO_3	1 liter
10 per cent HCl	1 liter
10 per cent NaOH	1 liter
10 per cent Lactic acid	1 liter
5 per cent $BaCl_2$	500 cc.
10 per cent Acetic acid	1 liter
Lime Water	1 liter
0.1 *N* NaOH	1 liter
0.1 *N* Lactic acid	1 liter

DESK EQUIPMENT

1. Please check your apparatus against this list and procure any missing items from the storeroom on order signed by the professor in charge.

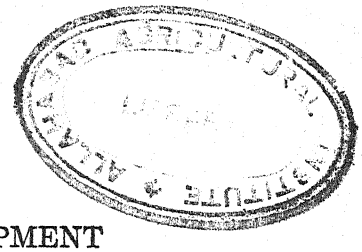
2. Keys will be furnished for your desks. If lost a charge of 75 cents will be made for each new key.

3. Procure several cheap towels.

4. Use a laboratory apron or coat; the saving in clothes and laundry bills will repay you.

5. Keep your apparatus clean, and keep it arranged in your desk and locker in an orderly manner.

6. An order book is provided to replace breakage. You are required to leave the desk at the end of the quarter with the permanent equipment just as you found it.



PERMANENT EQUIPMENT

No.	Item
2	Babcock milk test bottles
1	Babcock milk pipette
1	Babcock acid measure
1	pair dividers
14	beakers: one 150-cc., eight 250-cc., three 400-cc., two 800-cc.
2	burners with rubber tubing
2	1-pint milk bottles with rubber stoppers
1	½-pint milk bottle with rubber stopper
1	250-cc. narrow-neck bottle with rubber stopper
5	100–125-cc. glass-stoppered bottles
1	wash bottle, equipped
2	burettes, 0–25-cc., with pinchcocks
1	burette holder (double)
3	cylinders, graduated 10 cc., 50 cc., and 100 cc.
3	dishes, evaporating 100–250 cc.
1	funnel 1½″ diam., 6″ stem
2	funnels 3″ diam., 9″ stem
2	funnels 6″ diam., 12″ stem
1	funnel support, wooden
2	Erlenmeyer flasks 300 cc.
2	Erlenmeyer flasks 200 cc.
1	Erlenmeyer flask 100 cc.
2	volumetric flasks, 200 cc.
1	1-cc. pipette, graduated
1	10-cc. pipette, graduated
1	25-cc. volumetric pipette
1	100-cc. volumetric pipette
18	test tubes 30 cc.
5	test tubes 80 cc.
1	thermometer (−10 + 110° C.)

3 stirring rods, rubber-tipped
1 milk pan
1 mortar and pestle
2 fermentation tubes
1 single-hole rubber stopper for 30-cc. test tube
1 single-hole rubber stopper for 100-cc. Erlenmeyer flask
1 solid rubber stopper for 80-cc. test tube
2 tripods
2 rings for ring stand
1 ring stand
2 microscope slides and cover glasses
1 pair tongs
1 spatula
1 test-tube rack
1 file
2 wire gauze
1 clay triangle
1 test-tube holder
1 tube litmus paper (blue)
1 tube litmus paper (red)

TEMPORARY EQUIPMENT

1 cork stopper for 300-cc. Erlenmeyer flask
1 box matches
1 order book
50 filter papers 15 cm.
25 filter papers 25 cm.
1 test-tube brush
1 beaker brush

SPECIAL REQUIREMENTS FOR EACH EXPERIMENT

Experiment 1. — Westphal balances; lactometers; lactometer cylinders.

Experiment 2. — Milk testing 2.5, 4.5, and 6 per cent fat.

Experiment 4. — HCl solution, $pH = 4.6$, 100 cc. per student; 0.5 per cent $CuSO_4$.

Experiment 5. — 0.1 *M* calcium lactate; 0.1 *M* $CaCl_2$; 0.1 *M* Na_2HPO_4; 75 per cent alcohol.

Experiment 6. — McMichael viscosimeter.

Experiment 7. — Cenco-du Noüy tensiometer.

Experiment 8. — Phenolphthalein solution (1 per cent); methyl red solution; methyl orange; red litmus solution; blue litmus solution; hydrogen electrodes; saturated potassium oxalate solution, $pH = 6.5$; rennet; Clark's phosphate buffer $pH = 6.5$ (25 cc. per student).

Experiment 9. — 5 gallon jars, 1 for each 2 students; con. HCl; methyl red indicator; standard buffers, $pH = 4.6$ and 4.8; indicator comparator boxes; drain racks; toluene.

Experiment 10. — 0.1 *N* NH_4OH; 0.1 *N* KOH; 0.1 *N* HCl; $CaCO_3$; glacial acetic acid; 0.2 per cent H_3PO_4; NaCl; $MgSO_4$; $(NH_4)_2SO_4$; 0.2 molar borax (76 gms. $Na_2B_4O_7$, 10 H_2O per liter); formalin.

Experiment 11. — Milk of lime; $MgSO_4$; $(NH_4)_2SO_4$; saturated $(NH_4)_2SO_4$ solution $pH = 7$ (50 cc. per student); Almen's tannic acid reagent; powdered gelatin.

Experiment 12. — 75 per cent alcohol (50 cc. per student); 10 per cent NaCl.

Experiment 13. — Rennet; microscope; Fehling's solutions; taka-diastase; lactose; pure culture lactic acid organisms (5 cc. per student).

Experiment 14. — Pure lactic acid; alcohol; ether; $CHCl_3$; butterfat; lactic acid culture.

Experiment 15. — Nichrome or platinum wire; cobalt glass; NH_4CSN solution; potassium ferrocyanide solution; ammonium oxalate solution; 10 per cent Na_2HPO_4 solution; ammonium molybdate solution.

Experiment 16. — Butterfat; ether; $CHCl_3$; petroleum ether; acetone; ethyl alcohol; butyl alcohol; NH_4OH; alcoholic KOH, 4 per cent solution (50 cc. per student); Hübl's iodin solution (10–15 cc. per student); cream (50 cc. per student); formalin; steapsin powder; pure lactic acid; copper wire; con. HCl; 0.1 per cent ether solution of phloroglucin.

Experiment 17. — 3 per cent H_2O_2 (50 cc. per student); saturated solution guaiac in alcohol or acetone; 0.2 per cent H_2O_2; 2 per cent aqueous solution paraphenylenediamine; 5 per cent pyrogallic acid; 1 per cent H_2O_2; separator slime (5 cc. per student); $CHCl_3$; paraffin oil.

Experiment 18. — Fe_2Cl_6 crystals; alcohol; $(NH_4)_2SO_4$.

Experiment 19. — Microscope; saturated acetone solution of Sudan III; lactose; powdered gum acacia; saturated oil solution of Sudan III; fresh skim-milk powder.

Experiment 20. — Ultramicroscope; rennet; collodion; toluene; 2 per cent HCHO; 1 per cent gelatin solution.

Experiment 21. — Almen's tannic acid reagent; Fehling's solutions; ammonium molybdate; $AgNO_3$ solution; cobalt glass; K oxalate; guaiac solution; 0.2 per cent H_2O_2.

Experiment 22. — Butter; butterfat; gum damar; microscope; polarizing microscope.

Experiment 23. — Steapsin powder; copper lactate solution; sour cream and sweet cream ($\frac{1}{2}$ pint sour and 1 pint sweet per student); saturated ferrous lactate solution.

Experiment 24. — Aluminum cups; gasoline; standard $AgNO_3$ (see Experiment); K_2CrO_4 solution; butter.

Experiment 25. — Rennet.

Experiment 26. — $M/2$ $CaCl_2$; rennet.

Experiment 27. — Rennet; 5 per cent Na_2CO_3; 5 per cent NaH_2PO_4.

Experiment 28. — Saturated, neutral ($pH = 6.5$) potassium oxalate; $M/2$ $CaCl_2$; $M/2$ $MgCl_2$; $M/2$ $ZnCl_2$.

Experiment 29. — Rennet extract.

Experiment 30. — Gelatin powder; gum acacia powder.

Experiment 31. — Quartz sand; absorbent cotton; 1 per cent HCl; 0.5 per cent $CuSO_4$; $ZnSO_4$; MgO.

Experiment 32. — Fehling's reagents; Uffelmann's reagent (see Experiment).

Experiment 33. — NaCl; absorbent cotton.

Experiment 34. — Skim-milk powder; Fehling's reagents; Almen's tannic acid reagent; $M/2$ $CaCl_2$; rennet extract; guaiac solution; Storch reagent; 0.2 per cent H_2O_2.

Experiment 35. — Westphal balance or lactometer.

Experiment 36. — Standard NaOH and HCl (see Experiment); standard buffer, $pH = 4.6$; methyl red and phenolphthalein indicators; color comparator box.

Experiment 37. — Hortvet cryoscope; ether.

LABORATORY EXPERIMENTS IN DAIRY CHEMISTRY

CHAPTER I

PHYSICAL AND CHEMICAL PROPERTIES OF MILK

Experiment 1. — Specific Gravity

Determine the specific gravity of fresh whole milk and fresh skim milk with the Westphal balance and with an accurate lactometer. Read the temperature of the milks at which the gravity was taken. Change this value to ° F., using Table II in the Appendix. Correct the values found to the standard temperature of 60° F., using Table III and its footnote, in the Appendix. Compare the whole-milk and skim-milk values obtained by the two methods, at the observed and standard temperatures.

QUESTIONS

1. Explain the difference between the specific gravity of whole milk and that of skim milk.
2. What are the specific gravities of the principal constituents of milk, *i.e.*, fat, protein, lactose, mineral salts?
3. Why does the specific gravity of milk vary with the temperature?
4. Explain the principle involved in the determination of specific gravity by the Westphal balance and lactometer.

Experiment 2. — Calculation of Solids from Specific Gravity and Fat

Run a Babcock fat test and determine the specific gravity of three samples of milk of different fat content, which will

be furnished. From the percentage of fat thus found and the specific gravity corrected for the standard temperature of 60° F., calculate the following values for each sample.

Babcock's formulæ. —

(1) Plasma Solids

$$= 2.4703 \times (100 - f) \times \left(\frac{100\,S - Sf}{100 - 1.0753\,Sf} - 1 \right),$$

(2) Total Solids = Plasma Solids $+ f$,

(3) Total Solids $= 0.25\,L + 1.2\,f$

Richmond's Formula. —

(4) Total Solids $= 0.2625 \frac{L}{S} + 1.2\,f$

Hehner and Richmond's formula. —

(5) Total Solids $= 0.254\,L + 1.164\,f$

where f = percentage fat,
S = specific gravity,
L = Lactometer degrees.

Compare the results obtained in (2), (3), (4), and (5) for the three samples of milk.

QUESTION

Do the results show that there is any choice among the four formulæ when applied to the three types of milk, i.e., with high, low, or average fat content?

Experiment 3. — Calculation of Specific Volume

Calculate the specific volume of the three samples of milk used in Experiment 2, both at the observed temperature and at the standard temperature. Tabulate the results.

QUESTIONS

Do the data indicate any difference between milk fat and milk plasma so far as the effect of temperature on specific volume is concerned? If so, what difference is indicated?

Experiment 4. — Film Formation on Heating

(1) *Temperature of film formation.* — Place 50 cc. of cream, whole milk, and separated milk in each of three beakers. Heat each slowly in a water bath, and note the approximate temperature at which the film forms.

(2) *Character of film.* — Continue heating the skim milk until it boils. Remove the film with a stirring rod and watch it reform on continued boiling. Continue collecting the film until about half the milk has boiled away. Place the collected material in a beaker, and wash with distilled water by stirring the material up with several successive 25-cc. portions, pouring off the supernatant fluid carefully between washings. Repeat the washing with HCl solution with an acidity of $pH = 4.6$. Now dissolve the material in 50 cc. of 1 per cent NaOH. Test a portion of this solution for protein by the Biuret test.* Show that the protein is casein by adding dilute acid to the remainder of the solution, a few drops at a time, until coagulation occurs. Casein is the chief protein in milk that coagulates with acid.

Experiment 5. — Coagulation

(1) *Heat coagulation.* — Determine whether 50 cc. of milk will stand boiling for two minutes without coagulating. If so, determine the quantity of 0.1 N lactic acid which must be added to 50 cc. of the milk in order to secure coagulation on boiling for two minutes. In making this determination add the acid from a burette, 1 cc. at a time, in increasing

* The Biuret test is performed as follows: Take 5 cc. of solution in a test tube and add 5 cc. of 10 per cent NaOH. Make a dilute $CuSO_4$ solution containing not over 0.5 per cent $CuSO_4$ and add it drop by drop to the alkaline protein solution, mixing after each drop is added. If protein is present, 1 or 2 drops of the $CuSO_4$ will produce a violet color. Compare with the color of a check test, using water, alkali, and $CuSO_4$ solution in place of protein, alkali, and $CuSO_4$.

quantities, to separate 50-cc. portions of cold milk, until the quantity is found which causes the milk to coagulate when boiled. Now prepare three beakers each containing 50 cc. of milk. To one add 0.1 *M* calcium lactate solution; to another, 0.1 *M* $CaCl_2$ solution; to the third, 0.1 *M* calcium lactate and 0.1 *M* Na_2HPO_4 solution, the volume in each case being exactly that found to cause coagulation in the lactic acid test. Boil each sample for two minutes and note the coagulation except when Na_2HPO_4 was added.

The coagulation in the case of lactic acid, calcium lactate, and calcium chloride is due largely to an excess of calcium ions, which are rendered inactive by the PO_4 ions in the last test. Citrate or acetate ions will act in a like manner.

(2) *Alcohol coagulation.* — The alcohol coagulation of milk is recommended as a suitable guide for rejecting milk unsuited for condenseries. Prepare a set of samples identical with those which coagulated on boiling in part (1) of this experiment. Pipette 10 cc. of these into test tubes and add an equal volume of 75 per cent alcohol. Note whether coagulation occurs on mixing. Repeat the test on a sample of milk identical with the one that was protected from heat coagulation by Na_2HPO_4. Is it also protected from alcohol coagulation?

(3) *Acid coagulation.* — Add 10 per cent lactic acid solution, a drop at a time, from a pipette, to 50 cc. of fresh milk until coagulation occurs. Does this coagulated milk have the odor or flavor of " sour " milk? To what does naturally souring milk owe its flavor and odor?

Experiment 6. — Viscosity

(1) Determine the relative viscosity of (*a*) water, (*b*) whey, (*c*) fresh separated milk, (*d*) fresh whole milk, and (*e*) fresh 25 per cent cream at 20° C.

(2) Determine the viscosity of (*a*) normal 25 per cent cream and (*b*) the same cream after being homogenized at 20° C. Tabulate the data obtained in (1) and (2).

NOTE: This exercise is best performed with the McMichael or other torsion type viscosimeter, and may be performed by two students working together.

QUESTIONS

1. What is the effect of temperature on viscosity?
2. To what constituents is the viscosity of whey, separated milk, and whole milk largely due?
3. To what is the viscosity of cream largely due?

Experiment 7. — Surface Tension

Determine the surface tension of skim milk, whole milk, and 25 per cent cream at a uniform known temperature (approximately room temperature) using the Cenco-du Noüy tensiometer. Directions for operating the instrument must be secured from the instructor. The instrument is first adjusted to give a value of 73 at 22° C. for pure water. The scale then reads directly in dynes per centimeter for an unknown fluid. For unknown fluids at temperatures above 22° C., add 0.17 dynes for each degree above 22° C. For temperatures below 22° C., subtract 0.17 dynes for each degree below 22° C.

Experiment 8. — Reaction

(1) *By means of indicators.* — Add a few drops of each of the following indicator solutions to a dilute lactic acid and a dilute NaOH solution in test tubes; phenolphthalein, methyl red, methyl orange, red litmus, blue litmus. With the colors before you as checks, determine the reaction of fresh skim milk toward the same indicators. Tabulate the results.

QUESTION

Explain the difference in reaction of milk toward the different indicators.

(2) *True reaction.** — (*a*) Determine electrometrically the true reaction of fresh milk, using the hydrogen electrode. Express the results in terms of cH, pH, and °*S*.

QUESTIONS

1. What is true neutrality in terms of *cH*, *pH* and °*S*?
2. What is the character of the true reaction of fresh milk as judged from the determined value, i.e., acid, alkaline, or neutral?

(*b*) Determine the pH of milk to which very dilute lactic acid has been added until coagulation has begun and is distinct. Use 50 cc. of cold milk and 0.1 per cent lactic acid. Add the acid from a burette, drop by drop, with vigorous stirring.

(3) *Titratable acidity.* — (*a*) *Actual value.* — Pipette two 50-cc. quantities of milk into Erlenmeyer flasks of convenient size, add 25 cc. of distilled water and 0.5 cc. of phenolphthalein solution (1 per cent alcoholic solution) to each flask, and titrate to a faint permanent pink color, using 0.1 *N* alkali. Calculate result to 100 cc. of milk. (*b*) *Effect of removing calcium salts.* — Repeat the titration experiment, adding, in addition to 25 cc. of water, 1 cc. of a saturated potassium oxalate solution ($pH = 6.5$). Shake the oxalated milk gently by rotating the flasks, and let stand at least two minutes before titrating. Calculate the result to 100 cc. of milk. (*c*) *Effect of removing casein and calcium.* — Rennet removes the casein and from 50 to 60 per cent of the inorganic calcium, but does not affect the pH of milk. Determine the effect of such a procedure on the titratable

* This experiment can be utilized to give the students practice in determining hydrogen-ion concentration. The type of electrode used is immaterial but the Bailey electrode is recommended as giving very satisfactory results with milk.

acidity in the following manner. Measure 250 cc. of fresh skim milk into a beaker, warm to 40° C. in a water bath, add 1 cc. of fresh rennet extract, and keep the mixture at 40° C. until sufficient whey has diffused from the clot to make two 50-cc. portions of solution. Do not disturb the clot except to remove it gently from the sides and bottom of the beaker with a stirring rod. Add 25 cc. of water to each 50 cc. of whey and determine the titratable acidity as in (*a*). Calculate the results to 100 cc. of whey. (*d*) *Effect of adding neutral phosphates.* — Pipette 10 cc. of phosphate buffer solution, $pH = 6.5$ (see Table VIII in the Appendix), into each of two 50-cc. portions of skim milk. Add 25 cc. water and repeat the titration as in (*a*). Calculate the result to 100 cc. of milk.

QUESTION

What do the results of these experiments indicate as to the cause of the titratable acidity of fresh milk?

CHAPTER II

ISOLATION AND PROPERTIES OF MILK CONSTITUENTS

Experiment 9. — Preparation of Casein

Two students working together will prepare pure casein from 5 gals. of skim milk. The method to be employed is one that can be used for the commercial manufacture of casein. The product is called grain-curd casein. The directions are essentially those issued by the U. S. Bureau of Dairying for commercial practice. The principles involved are (*a*) the removal of the calcium from the calcium caseinates through the addition of acid until the reaction of the milk is at the isoelectric point of casein, (*b*) the control of the reaction by means of an indicator, (*c*) the further control of the character of the casein curd by strict attention to the temperature of the milk, and amount of stirring, and (*d*) the maintenance of the desired consistency during purification by means of wash water the reaction of which is adjusted to the acidity at which casein exists in the isoelectric state.

Possible difficulties and causes:

A. *Lump curd.*
   1. Too high temperature.
   2. Insufficient stirring during addition of acid.

B. *Soft curd.*
   1. Insufficient acid.
   2. Insufficient stirring.

C. Finely divided curd.

1. Too low temperature.
2. Too vigorous stirring during addition of acid.

The acid used to precipitate the casein. — One part of hydrochloric acid containing 31.45 per cent HCl (sp. gr. 1.15 or 20° Baumé) is diluted with 8 parts of water and mixed thoroughly. To prepare 1.16 sp. gr. HCl from stock concentrated HCl, dilute 29.3 cc. with 5.2 cc. water. To prepare the acid for precipitating, dilute the resulting mixture with 276 cc. of water.

Indicator solution. — This will be furnished. It is prepared by dissolving 0.04 gram of methyl red powder in 100 cc. of 95 per cent ethyl alcohol. The indicator should be protected from the light.

Indicator standards. — (*a*) Standard $pH = 4.6$. Add 0.08 cc. of methyl red indicator solution to each of two 10-cc. portions of standard buffer of $pH = 4.6$ (this will be furnished) in test tubes. Stopper and retain for comparison with whey. The 4.6 buffer is a mixture of 62.5 cc. $M/5$ KH phthalate and 30.38 cc. $N/10$ NaOH diluted to 250 cc. in a standard flask. The buffer will keep for several weeks. (*b*) Standard $pH = 4.8$. Add 0.08 cc. of indicator to each of two 10-cc. portions of standard buffer of $pH = 4.8$ (this will be furnished) in test tubes. Stopper and retain for comparison with wash water throughout the entire washing procedure. The 4.8 buffer is a mixture of 62.5 cc. $M/5$ KH phthalate and 44.25 cc. $N/10$ NaOH diluted to 250 cc. in a standard flask.

The milk. — The skim milk should be as free from fat as possible, and sweet. It should also be free from foam, and it must be raw. For pasteurized and sweet cream buttermilk, see *Journal of Industrial and Engineering Chemistry*, **13**, 510, 1921.

Precipitating the casein. — Warm the skim milk to 34.5°–35° C. (never over 35.5° C.), checking with an accurate thermometer. (The precipitating temperature is 94° F.) Now, slowly and continuously stirring, pour in the " precipitating acid " in a small steady stream until the milk " breaks," giving a fairly clear whey. Test the acidity at this point, using 0.08 cc. of methyl red and 10 cc. of whey. Save this test for reference. The correct method for making this color comparison is described in detail in Experiment 36. Allow the curd to settle and pour off about two-thirds of the whey, preferably through the cheesecloth drain to catch small particles of casein. The removal of the bulk of whey at this point is not necessary, but reduces the amount of acid required to finish the operation. (See below.)

Second addition of acid. — Stir up, thoroughly but not rapidly, the curd and whey remaining in the jar, in order to break up any clumps. Now add more " precipitating acid," a few cubic centimeters at a time, mixing thoroughly and testing 10-cc. portions of the whey after each addition of acid, until an acidity of $pH = 4.6$ is obtained. This is the " isoelectric " point of casein, or the acidity at which casein exists in the freest state. The use of the indicator is based on this chemical fact and gives a control of the manufacture of casein which would not be possible otherwise. *The acidity should never be carried past* $pH = 4.6$. The casein at this stage is firm and grainy and rather brittle, if it has been properly prepared and if the precautions as to temperature, proper stirring, and acidity have been observed.

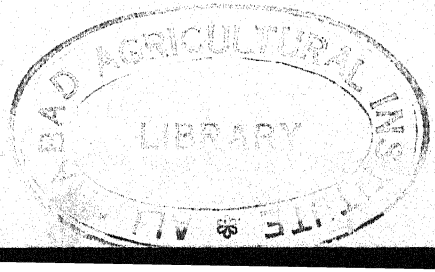
Wash water. — The casein curd is not washed with water but with HCl of a strength $pH = 4.8$ to 5.0, which is prepared by adding the acid used for precipitation to water, until it shows the proper color with methyl red indicator. The use of wash water with the proper strength of acidity is *very important* in the manufacture of good casein, as it dis-

solves the adhering mineral salts, thereby reducing the ash content of the final product. It also prevents a redispersing of the casein, thus insuring that the curd will retain its original " grain."

Washing the curd. — Drain off the remainder of the whey through the cheesecloth. Wash the casein three times with the " wash water," the acidity of which has been checked with $pH = 4.8$, covering the curd with the wash solution, stirring up gently and pouring off the wash solution through the cheesecloth drain after each addition of " wash water." The " wash water " should never be above the precipitating temperature of the casein (94° F.). (In large-scale manufacture only one washing in the vat is necessary, followed by one on the drain rack.) Finally, transfer the curd to a drain rack and wash once with a liberal amount of cold distilled water.

Purity of curd. — The casein may now be regarded as a high-grade " technical " product. The next step in the commercial production of casein would be to dry it on screens in a chamber through which a current of warm air is passed. Chemically speaking, the casein is not pure but could serve as the starting point for purification. Methods for preparing pure casein are described by Van Slyke and Baker. (N. Y. Expt. Sta. Tech. Bul. **65**, 1918) and by Van Slyke (Proc. World's Dairy Congress, 1923, p. 1145).

Preserving the casein. — The moist casein prepared by the various members of the class will be combined, pressed as dry as possible in a cheese press, and the moisture content determined. Each member of the class will preserve 100–150 grams of the moist casein in a well-stoppered, wide-mouth bottle on the bottom of which has been placed a layer of cotton saturated with toluene. *Keep in ice box.*



Experiment 10. — Properties of Casein

(1) *Compounds of casein with alkalies and acids.* — Weigh out the equivalent of three approximately 2-gram portions of dry casein, using the moist product prepared in the preceding experiment; grind* each for a few minutes in a mortar with 16-cc. portions of $N/10$ NaOH, $N/10$ NH_4OH, and $N/10$ KOH respectively, and transfer the mixtures to large test tubes. In a like manner treat the equivalent of 1-gram portions of dry casein with 20 cc. of clear lime water (saturated $Ca(OH)_2$ solution) and 15 cc. of glacial acetic acid. Dilute the mixtures containing NaOH, NH_4OH, and KOH with an equal volume of water. Make the following observations and tests on each tube: (1) appearance (clear, opalescent, or milky), (2) extent of solution, (3) reaction of samples containing NaOH, NH_4OH, KOH, and $Ca(OH)_2$ to litmus and phenolphthalein, (4) effect of heating to boiling, (5) effect of adding an equal volume of $N/10$ HCl to the tests containing NaOH, NH_4OH, KOH, and $Ca(OH)_2$.

(2) *Reaction of casein with $CaCO_3$.* — Make a thin paste of 5 grams of moist casein by grinding with the least possible amount of water in a mortar. Transfer to a 30-cc. test tube and add 5 cc. of a *water suspension* of $CaCO_3$. Shake thoroughly and stopper the tube with a rubber stopper which has a piece of bent glass tubing extending just below the bottom of the stopper, the rest of the glass tube extending over the side of the test tube at a slight angle to a length of about 6 inches. Insert the long end of the tube into a fermentation tube containing clear *lime water* with the end of the tube under the lime water solution and beneath the long arm of the tube.

* Success in making these dispersions depends on the addition of the alkalies in small aliquots with the vigorous trituration of the mixture after each addition of reagent.

QUESTIONS

1. What effect has the escaping gas on the lime water solution?
2. What is the nature of this gas and of the reaction which has taken place between the casein and the $CaCO_3$, and also between the gas and the lime water?

(3) *Preparation of casein milk (calcium caseinate solutions).* — Using the moist casein, grind the equivalent of 6 grams of dry casein with 0.16 gram of $Ca(OH)_2$ powder and a few cubic centimeters of water until a smooth paste is obtained. Dilute slowly to a volume of 100 cc., using distilled water and constant trituration. Transfer to a beaker and neutralize very carefully to litmus as follows: Place a piece of sensitive litmus paper in the milk and very slowly drop 0.2 per cent H_3PO_4 solution into the milk from a burette while the milk is being *vigorously* stirred, until the litmus shows the milk to be neutral. (If possible run the *p H* of this milk, it being desired to have it at *p H* of 6.3 to 6.5.) Dilute the milk to a final volume of approximately 200 cc. so that it will have a casein concentration equivalent to about 3 per cent. Test with litmus to insure proper *p H*.

(4) *Properties of calcium caseinate solution.* — Compare the properties of skim milk and calcium caseinate solution in the following respects, using equal quantities of each: (*a*) *Film formation.* — Heat 50-cc. quantities of the skim milk and calcium caseinate solutions slowly to boiling, noting the formation of film which can be removed and will reform. Is there any difference between milk and calcium caseinate solution in this respect? (*b*) *Salting out of calcium caseinate.* — Add 10 grams of NaCl, 10 grams of $MgSO_4$, and 20 grams of $(NH_4)_2SO_4$ crystals respectively to 25-cc. portions of milk and calcium caseinate solution in large test tubes, and shake gently until no more of the crystals dissolve. Note precipitation of protein. Determine whether the protein has been rendered permanently insoluble by

diluting the tests with 100–150 cc. of distilled water. (*c*) *Effect of acid.* — Add dilute acid to 25-cc. portions of skim milk and calcium caseinate solutions until coagulation occurs. Determine whether dilution with distilled water affects this precipitate. (*d*) *Effect of rennet.* — Warm the remainder of the calcium caseinate solution and an equal volume of skim milk to 40° C. Add 1 cc. of dilute rennet solution (1 cc. of fresh rennet extract in 10 cc. H_2O). Compare the results.

(5) *Casein glue.* — Casein glue may be made by a large number of formulæ, most of which are covered by patents. A good glue may be made by dissolving casein in borax. Weigh out the equivalent of 5 grams of dry casein and add it to 35 cc. of 0.2 molar borax (76.32 grams of $Na_2B_4O_7$, 10 H_2O per liter) at 30° C., with vigorous stirring. Let stand thirty minutes, stirring vigorously at five-minute intervals. Test the adhesive properties of the glue on wood.

(6) *Hardened casein plastic.* — Heat 100 cc. of skim milk to 50° C., add 10 per cent acetic acid until the casein precipitates in a lump. Remove and press between the fingers until as free as possible from whey. Now knead the lump for a few minutes under the *hot*-water tap until it becomes tough and elastic. Wipe dry and place in a beaker and cover with formalin. Let stand until next period. Wash with water and examine the product, noting particularly the increase in hardness. Dry in an oven at 60–80° C. and note the bone-like character of the product. The general principles involved here are used in the manufacture of artificial ivory.

Further properties of casein are studied in Chapter III.

Experiment 11. — Lactalbumin and Globulin

(1) *Preparation of milk serum.* — Measure 400 cc. of fresh skim milk into a large beaker. Add 10 per cent lactic acid solution slowly from a burette with vigorous stirring with a rubber-tipped stirring rod, until a flocculent precipitate forms. Allow the precipitate to settle and decant the supernatant fluid through a filter. The casein may be discarded. Add a few drops of phenolphthalein solution to the filtrate and carefully neutralize to a faint pink with a thin "milk of lime" (suspension of $Ca(OH)_2$ in water). Filter off the precipitate through a large fluted filter. This precipitate is called the neutralization precipitate of milk serum and consists largely of phosphates. Add dilute (1 per cent) HCl to the filtrate until neutral to litmus. The following tests require 300 cc. of filtrate.

(2) *Salting out the globulin.* — Dilute 50 cc. of serum with an equal volume of water and saturate the solution with $MgSO_4$ crystals (about 40 grams will be required). The precipitate is globulin. All animal globulins are salted out of their neutral solutions on saturation with $MgSO_4$.

(3) *Salting out the lactalbumin and globulin.* — Dilute 50 cc. of serum with an equal volume of water and completely saturate with $(NH_4)_2SO_4$ crystals (about 80 grams will be required). The precipitate which forms is a mixture of lactalbumin and globulin. All animal albumins and globulins are salted out of their neutral solutions on complete saturation with $(NH_4)_2SO_4$.

(4) *Separating the globulin and lactalbumin.* — Dilute 50 cc. of serum with an equal volume of a saturated $(NH_4)_2SO_4$ solution that is neutral to litmus. The precipitate is globulin, which is salted out of neutral solutions on half saturation with $(NH_4)_2SO_4$. Filter off the globulin and add dilute (5 per cent) H_2SO_4 solution to the filtrate, drop by drop,

with stirring, until a heavy precipitate forms. The precipitate is lactalbumin, which is salted out on half saturation of its solutions with $(NH_4)_2SO_4$ when the reaction is at the isoelectric point of the albumin. Determine approximately the *pH* of the filtrate from the albumin, using methyl red and the standard indicator solutions *pH* = 4.6 and 4.8, which were used in the preparation of casein (Experiment 9).

Note the relative amounts of precipitates in this experiment, indicating the relative quantity of lactalbumin and globulin in average cow's milk.

(5) *Heat coagulation of serum proteins.* — Dilute 50 cc. of serum with an equal volume of water, add 0.3 cc. of 10 per cent acetic acid, and heat slowly to 75° C. in a water bath. Record the temperature of first cloudiness and the temperature of coagulation. Observe the stages in the heat coagulation of the proteins in this test, i.e., (1) the denaturing as shown by the cloudiness, (2) the agglutination of the denatured colloidal particles. Now raise the temperature of the solution to boiling and boil for five minutes. Filter off the coagulated material while the solution is still hot, in order to facilitate filtration. Test the filtrate for protein, using the acetic acid-ferrocyanide test, as follows: To 10 cc. of water-clear filtrate add 1 cc. of 10 per cent acetic acid, and then add, drop by drop, with shaking after each addition, a 5 per cent solution of potassium ferrocyanide. A positive test for protein is shown by a distinct cloudiness.

QUESTIONS

1. What proteins are coagulated by heat in this experiment?
2. Is coagulation complete by this means?

(6) *Compounds of serum proteins with tannic acid.* — To 50 cc. of serum add 25 cc. of Almen's tannic acid reagent (2 per cent solution of tannic acid in 50 per cent alcohol

containing 10 per cent acetic acid). Let stand for half an hour and filter. Test the filtrate for protein with the acetic acid-ferrocyanide test. In this case no acetic acid need be added since the Almen's reagent contains it. Compare with Part (5).

(7) *Protecting against heat precipitation.* — Dilute 50 cc. of serum with 100 cc. of freshly prepared 6 per cent gelatin solution. Heat to boiling.

QUESTIONS

1. What is the effect of the protective colloid (gelatin) on the agglutination of heat-denatured lactalbumin and globulin?
2. Does the result explain why boiled milk shows no precipitate of albumin?

Further properties of lactalbumin are given in Chapter III.

Experiment 12. — Alcohol-Soluble Protein

Transfer 10 to 15 grams of the moist casein from Experiment 9 to a 200-cc. Erlenmeyer flask, add 50 cc. of 75 per cent alcohol, and heat to boiling in a water bath. Filter the hot extract so as to obtain as clear a filtrate as possible. On cooling, the solution may become cloudy if sufficient alcohol-soluble protein is present. Heat the filtrate, if cloudy, to ascertain if the cloudiness is due to alcohol-soluble protein, which is especially soluble in hot alcohol. Pour the clear, hot solution into 100 cc. of distilled water. The precipitate which settles is the alcohol-soluble protein. Settling can be facilitated by adding 2.5 cc. of 10 per cent NaCl solution. Allow to stand until settled. Decant the water and test the solubility of the precipitate in NaOH. Make a biuret test on a portion of this solution and determine the effect of acidifying the remainder with HCl.

QUESTIONS

1. How do the properties of casein and the alcohol-soluble protein resemble each other?

2. Explain the presence of the alcohol-soluble protein in the casein preparation.

Experiment 13. — Preparation and Properties of Lactose

(1) *Preparation.* — Remove the casein from 500 cc. of skim milk with rennet, by warming to 40° C., adding 1 cc. fresh rennet extract, and holding at 40° C. until the whey has been expelled. After the clot has become quite firm, it can be pressed gently with the hands so as to recover 400–500 cc. of whey. Add 1 cc. of 3 per cent HCl for each 50 cc. of whey. Heat to boiling and boil for ten to fifteen minutes. Filter off the coagulated proteins, make the filtrate neutral to litmus, using milk of lime, and evaporate to a sirup in an evaporating dish on a boiling water bath. *Do not boil it down to a sirup over a free flame.* If a precipitate forms during concentration, filter it off and proceed with the evaporation. Let the final sirup stand until the next laboratory period. If the concentration has been carried to the proper point, the sirup will contain lactose crystals. There will also be crystals of inorganic salts. In order to be able to identify the lactose crystals, prepare the following before setting the sirup aside: — Dissolve 5 grams of lactose powder in 10 cc. of boiling water and allow to stand with the whey sirup.

(2) *Properties.* — (*a*) *Form of crystals.* — Examine the sirup and also the prepared lactose under the microscope, to determine whether the sugar has crystallized. Note the wedge-like form of the lactose crystals, and their hard, sandy consistency. (*b*) *Reducing action.* — Test the reducing action of the sirup toward Fehling's solution as follows. Mix 2 cc. each of Fehling's solutions Nos. 1 and 2

in a test tube, heat to boiling, and add a few drops of the sirup. Continue the boiling for a minute until the precipitate forms. If none forms, add more of the sirup.

QUESTIONS

1. What is Fehling's solution?
2. What is the nature of the precipitate which forms in the test?
3. Which sugars reduce Fehling's solution and which do not?

(*c*) *Alcoholic fermentation.* — Divide the remainder of the sirup into two parts. Rub up each part in a mortar with a small piece of compressed yeast, and in addition add 0.1 gram of takadiastase to one test. Transfer each test to a saccharometer (fermentation tube) and let stand in a warm place in a beaker until the next laboratory period. If alcoholic fermentation occurs, CO_2 will collect in the closed portion of the tube.

Compare and explain the results of the two tests.

(*d*) *Acid fermentation.* — Prepare three clean 100–150 cc. bottles, measure into one bottle 75 cc. of 5 per cent lactose solution, into another 75 cc. of approximately 5 per cent lactose plus 15 cc. of boiled skim milk, and into the third 75 cc. of boiled skim milk. Add 5 cc. of lactic acid culture (buttermilk starter) to each with a pipette. Mix the solutions, and pipette out 25 cc. of each mixture at once into a clean flask and titrate its acidity with standard 0.1 *N* NaOH solution. Set the remainder of the solutions aside in your desk and repeat the titrations at the next laboratory period. Calculate the initial and final acidities in terms of cubic centimeters of 0.1 *N* NaOH required for 100 cc. of solution.

QUESTION

How would you explain the relatively low acid development in the lactose solution in comparison with that of the milk and that to which a small amount of milk was added?

Experiment 14. — Lactic Acid

(1) *Solubility.* — Determine the solubility of lactic acid in water, alcohol, ether, chloroform, and melted butterfat by placing 10 cc. of each solvent in test tubes and adding to each a few drops of pure lactic acid and mixing. Record results.

(2) *Extraction from water by ether.* — Pipette 50 cc. of 0.1 *N* lactic acid solution into a 100–125-cc. glass-stoppered bottle. Measure 50 cc. of ether into the bottle, stopper tightly, and shake gently at intervals for fifteen minutes. Keeping the bottle tightly stoppered, let stand for one-half hour so that the ether and water layers will separate as completely as possible. Pipette out 25 cc. of ether, transfer to an Erlenmeyer flask, dilute with 50 cc. of neutral alcohol, and determine the amount of 0.1 *N* lactic acid recovered in the ether by titration with 0.1 *N* alkali, using phenolphthalein as indicator. Calculate the percentage of lactic acid extracted by the ether, bearing in mind that the amount titrated represents only one-half of that extracted by the ether.

(3) *Amount of free lactic acid in sour milk.* — Measure 70 cc. of pasteurized skim milk into a 100-cc. glass-stoppered bottle. Add a few drops of pure culture of lactic-acid-forming bacteria. Pipette out 10 cc. and determine its titratable acidity. Convert the result to grams of acid per 100 cc. of milk. Allow the milk to stand in a warm place until the next laboratory period. Determine the total titratable acidity on 10 cc. of the sour milk after shaking the clabbered milk to break up the clot and secure a uniform consistency. Convert the result to grams of acid per 100 cc. milk. Now add 50 cc. of ether to the remaining 50 cc. of milk and carry out the extraction and lactic acid determination as in Part (2) of this experiment. It may be

necessary to centrifuge the mixture of sour milk and ether in order to secure 25 cc. of ether for titration.

From the results secured in Parts (2) and (3) of this experiment, calculate the approximate percentage of total acid formed in the sour milk which is present as free lactic acid, ignoring the sp. gr. of the milk. The following formulæ will give the result:

(1) Final acidity of milk − initial acidity = total acid formed.
(2) Acid extracted by ether from sour milk × 4 = total acid extractable.
(3) Total acid extractable ÷ percentage extractable as found in Part (2) = total free acid.
(4) Total free acid ÷ total acid formed = percentage formed present as free acid.

Experiment 15. — Mineral Elements in Milk Ash

Add a few drops of strong acetic acid to 50 cc. of skim milk in a 100-cc. porcelain dish, evaporate to dryness, and ignite to a gray ash over the Bunsen flame. Dissolve the ash in 50 cc. of hot 10 per cent HCl solution, filter, and use the filtrate for the following tests:

(1) *Sodium.* — Hold a piece of platinum or nichrome wire in the blue Bunsen flame until it imparts little or no color to the flame. Dip the wire in the solution and hold it in the flame again. If sodium ions are present the flame will show a bright yellow color.

(2) *Potassium.* — Obtain a piece of "cobalt glass" from the instructor. Test for potassium as you did for sodium, but view the flame through the glass. If potassium is present the flame will appear lavender or violet.

(3) *Iron.* — Place 5 cc. of the acid extract of the milk ash in each of two test tubes. To one add an equal volume of

ammonium thiocyanate solution; a red color indicates the presence of iron. To the other add a little potassium ferrocyanide solution; a precipitate of Prussian blue or a Prussian blue color indicates the presence of iron. Run a blank test with either reagent on 5 cc. of the HCl solution used to extract the milk ash. Does milk contain appreciable amounts of iron?

(4) *Calcium.* — Add NH_4OH to 5 cc. of the acid extract in a test tube until it is slightly alkaline to litmus. Now add ammonium oxalate solution. A white precipitate of calcium oxalate indicates the presence of calcium.

(5) *Magnesium.* — Place 5 cc. of the acid extract of the milk ash in a test tube and make neutral with NH_4OH. Add 5 cc. of a solution of disodium phosphate, Na_2HPO_4, make strongly alkaline with NH_4OH, and note the formation of a white precipitate of magnesium ammonium phosphate if magnesium is present.

(6) *Sulfur.* — Add 1 cc. of $BaCl_2$ solution to 5 cc. of acid extract; note the formation of a white precipitate of $BaSO_4$ if sulfates are present.

(7) *Phosphorus.* — Carefully neutralize 5 cc. of acid extract, using NH_4OH. Add a few drops of HNO_3. Heat to boiling, and add ammonium molybdate to the hot solution. A yellow precipitate of ammonium phosphomolybdate indicates the presence of phosphorus.

Experiment 16. — Milk Fat

(1) *Solubility.* — Test the solubility of butterfat in ether, chloroform, petroleum ether, acetone, ethyl alcohol, and butyl alcohol, by placing 5 cc. of each reagent in a dry test tube and adding 2 cc. of melted butter fat and mixing gently. Record the results. Heat the alcohol test to boiling by immersing in boiling water. Result? Now cool under cold-water tap. Result?

(2) *Extraction from milk.* — Place 5 cc. of whole milk in a test tube of about 80 cc. capacity, add 0.5 cc. of concentrated NH_4OH, and mix thoroughly. Add 5 cc. of 95 per cent alcohol and mix well. Now add 10 cc. of ether and shake vigorously with the thumb held tightly over the mouth of the test tube or with the test tube tightly corked. Continue the shaking for about one-half minute. Now add 10 cc. of petroleum ether and repeat the shaking. Let stand for ten or fifteen minutes. Pour off the clear supernatant fluid (if not quite clear, pour off through a small filter) into a small dry beaker. Evaporate to dryness on a water bath. The principle of this method of extracting fat is the same as that used in the Röse-Gottlieb and Mojonnier methods for the quantitative extraction of fat from milk.

The function of the various reagents used in this method is as follows: The NH_4OH converts the calcium caseinates to more highly dispersed ammonium caseinates. The emulsion of fat in the ammonium caseinate is readily broken by the alcohol so that the ether can extract the fat. The petroleum ether is added so that the extract will contain the least possible amount of water, water being miscible to some extent with ether but not with petroleum ether.

(3) *Saponification.* — Add 5 cc. of melted butterfat to 50 cc. of 4 per cent KOH solution in 95 per cent alcohol in an Erlenmeyer flask. Heat in *water bath,* allowing the alcohol to simmer until it has entirely evaporated and no odor of alcohol is detected. Dissolve the soap in 50 cc. of water with the aid of heat. Note its soapy odor and other soapy properties.

(4) *Liberation of fatty acids.* — Add 40 cc. of 10 per cent H_2SO_4 to the soap. Note the cloudy precipitate of water-insoluble fatty acids.

(5) *Presence of butyric acid.* — Heat the acidified soap

solution on the water bath. Note the strong odor of butyric acid.

(6) *Presence of oleic acid in water-insoluble fatty acids.* — Continue heating the soap solution until the fatty acids rise as a clear layer on the surface. Now chill the solution in ice water until the fatty acids have set in a solid cake. Remove carefully, dry quickly between filter papers, and dissolve in 10 cc. of $CHCl_3$ in a dry test tube. Add Hübl's* iodin solution, a drop at a time, to the $CHCl_3$ solution, allowing the iodin color to disappear before adding more iodin. The oleic acid in the material used should absorb several cubic centimeters of Hübl's iodin solution, showing the presence of unsaturated fatty acid.

(7) *Iodin absorption by glycerides of butterfat.* — Repeat the iodin absorption experiment of Part (6) of this experiment, using 1 cc. of melted butterfat instead of the free fatty acids. Note that the occurrence of oleic acid in the form of a glyceride does not interfere with the absorption of iodin.

(8) *Fractional crystallization of glycerides.* — Allow a test tube full of melted butterfat to stand at room temperature until it appears to have solidified. This may require several hours, depending on the original temperature of the fat. Pour the apparently solid fat into a dry, fluted filter with a capacity about equal to the volume of fat. Use a dry funnel and place a dry test tube under it to catch the filtrate. Allow the filtration to continue until the next laboratory period.

QUESTION

Is butterfat a chemical compound or a mixture of chemical compounds?

* Hübl's iodin solution is a mixture of equal parts 5.4 per cent alcoholic solution of iodin and 6.0 per cent alcoholic solution of $HgCl_2$, each in 95 per cent alcohol. When used for quantitative determination of the iodin value of fat, the mixture is allowed to stand twenty-four hours before using.

(9) *Hydrolysis by enzyme.* — Measure 50 cc. of cream into a 200-cc. Erlenmeyer flask. Add 0.08 cc. of formalin (40 per cent solution of formaldehyde) and 1 gram of steapsin powder dissolved in 2–3 cc. of water. Stopper with a cotton plug and set aside in a warm place (incubator at 38° C., if possible) until the next laboratory period. Examine the flask for hydrolysis of the butterfat as indicated by the liberation of characteristic fatty acids. The fat has become rancid (hydrolytic rancidity).

(10) *Oxidation by metallic salts.* — Add a very small drop of pure lactic acid to 10 cc. of melted butterfat in a dry test tube, mix, and then place a piece of bright copper wire in the tube. Heat in a boiling water bath and examine from time to time to note (*a*) bleaching and discoloration, (*b*) tallowy, oxidized odor. If the reaction does not occur during the laboratory period, let stand until the succeeding period.

(11) *Test for oxidized fat (Kreis test).* — This test, often referred to as a test for rancidity, is a test for only one type of rancidity, namely, oxidative rancidity. Perform the test on the oxidized butterfat and on a sample of normal untreated butterfat for comparison. Transfer the oxidized fat to a large test tube (50 cc. capacity) and prepare a similar tube of fresh unoxidized fat. Add 10 cc. of conc. HCl (sp. gr. 1.19) to each tube. Close the tube tightly with a *rubber* stopper and shake vigorously for thirty seconds. Ten cubic centimeters of 0.1 per cent ether solution of phloroglucin are next added, and the tube closed and shaken as before. It is then allowed to stand. If the fat has become oxidized, a red or pink color will appear in the acid layer. The depth of the color is roughly proportional to the degree of oxidation. A faint orange or yellow tint is not regarded as a positive test. The test is positive in fats which have not yet begun to show the tallowy decomposition by taste or odor.

Experiment 17. — Milk Enzymes

(1) *Catalase.* — Milk contains an enzyme that greatly accelerates the decomposition of H_2O_2 with the liberation of molecular oxygen. There are two sources of the catalase in cow's milk, the mammary gland and bacteria, but the greater part in ordinary market milk is of bacterial origin. (*a*) *Fresh milk.* — Measure 50 cc. of fresh milk into a 100-cc. Erlenmeyer flask provided with a rubber stopper through which is passed a glass tube the other part of which is bent so that its end can be inserted into the upright arm of a fermentation tube. Fill a fermentation tube full of distilled water and insert the glass tube carrying the rubber stopper which is to be used for the flask containing the milk. Now add 25 cc. of 3 per cent H_2O_2 solution to the 50 cc. of milk which has been warmed to 35° C., carefully connect the flask at once with the fermentation tube and let stand at 35° C. until the gas has ceased to be evolved. Calculate the catalase activity of the milk in terms of the number of cubic centimeters of oxygen liberated from 100 cc. of milk. (*b*) *Heated milk.* — Repeat the catalase test on milk which has been heated to 80° C. and then cooled to 35° C.

(2) *Peroxidase.* — Fresh milk contains an enzyme that is capable of transferring oxygen from hydrogen peroxide to certain oxidizable compounds, the oxidation of which gives rise to a characteristic color. Some of the various compounds used for this purpose are guaiac, paraphenylenediamine, ortol, amidol, benzidine, pyrogallic acid, etc. (*a*) *Guaiac test.* — Place 5 cc. of milk in a test tube, let a few drops of saturated alcoholic or acetone guaiac solution flow down the side of the test tube on to the surface of the milk, and follow this with a few drops of 0.2 per cent H_2O_2. A positive test is indicated by the development of a blue color. If the color develops before the addition of the

peroxide it indicates that the guaiac solution itself contains sufficient peroxide material for the reaction. (*b*) *Storch test.* — Add a few drops of 0.2 per cent H_2O_2 solution to 5 cc. of raw milk, previously warmed to 38° C. Follow at once with a few drops of freshly prepared 2.0 per cent of paraphenylenediamine. Shake the tube. If peroxidase is present an intense blue color develops at once. A bluish-gray color at once or after half a minute is a negative test. (*c*) *Purpurogallin test.* — Place 2 cc. of fresh skim milk in a test tube, add 10 cc. of distilled water, 2 cc. of freshly prepared 5 per cent solution of pyrogallic acid, and 2 cc. of 1 per cent H_2O_2 solution. Shake well, and pour paraffin oil on the surface to form a thin layer, so as to protect the solution from the air. A red precipitate of purpurogallin, which slowly increases on standing, indicates the presence of peroxidase. (*d*) *Effect of heat on peroxidase.* — Repeat the peroxidase tests on milk that has been heated to 80° C. (*e*) *Effect of acid on peroxidase reaction.* — The peroxidase reaction of raw milk with paraphenylenediamine (Storch test) is inhibited by a *pH* of 4.5, so that it is not applicable to curdled milk. The test with guaiac, however, is not so affected, showing that the effect of the acid reaction is not on the peroxidase but on the Storch reagents. Milk or cream that has undergone a yeasty fermentation, however, no longer gives the test with guaiac.

Repeat the Storch and guaiac tests on milk that has had lactic acid added until the casein has precipitated. Now add dilute NaOH to some of the coagulated milk until it is again neutral to litmus paper. Repeat the Storch and guaiac tests on the neutralized milk.

(3) *Galactase.* — Milk contains an enzyme that is capable of causing the hydrolysis or digestion of its own proteins. It is found especially concentrated in separator slime.

Put 100 cc. of fresh skim milk in a bottle, add 2.5 cc. of

chloroform and about 5 cc. of separator slime. Shake the bottle thoroughly and stopper tightly. Set aside in an incubator at 38° C. and observe any changes that take place. Examine the bottle at each successive laboratory period until evidences of digestion appear.

Experiment 18. — Pigments of Milk

(1) *Carotinoids in butterfat.* — Warm 5 cc. of natural-colored butterfat in a test tube and add a very small crystal of ferric chloride. Keep warm and observe the change in color to a bright green. If too large an amount of ferric chloride is added the green color will not appear. Shake vigorously with 25 cc. of hot 95 per cent alcohol. Let stand until the fat has separated. It should be colorless.

Ferric chloride is reduced by carotinoids to green ferrous chloride and the carotinoids are oxidized and rendered colorless.

(2) *Whey pigment.* — This is due to a substance called lactochrome. It causes the greenish-yellow color of milk serum and milk whey. The substance is chemically closely related to urochrome, the cause of the color of urine. When fresh milk is rapidly freed from casein and heat-coagulable proteins, the filtrate still retains the greenish-yellow color. On saturation of this filtrate with solid $(NH_4)_2SO_4$ and addition of an equal volume of strong alcohol, the lactochrome will rise to the top in the alcohol layer. This experiment is optional and need not be carried out unless desired.

CHAPTER III

CHEMICAL AND PHYSICO-CHEMICAL STATE OF MILK CONSTITUENTS

Experiment 19. — Coarse Dispersions

The fat of milk exists as a coarse emulsion of liquid globules readily visible under the microscope. The diameter of the globules varies between extremes of 0.1 to 22.2 microns, but the greater number are from 2 to 7 microns.

(1) *Microscopic appearance.* — Dilute 5 cc. of whole milk with 250 cc. of water at a temperature of 35° C. Put a drop of the diluted fluid on a microscope slide, place a cover glass over it, and examine under the microscope. Note the Brownian movement of the smaller globules.

(2) *Staining the fat globules.* — Add 1 cc. of a saturated acetone solution of Sudan III to 5 cc. of whole milk in a test tube. Observe the proportions carefully. Mix gently and let stand for about ten minutes. Now pour the stained milk into 250 cc. of warm water (35° C.) and examine under microscope as in Part (1). Note that the fat globules have taken up the red fat stain and now stand out clearly.

(3) *Artificial emulsions.* — (*a*) *An unstable emulsion.* — Five grams of milk sugar are placed in a mortar and 7.5 cc. of water are added gradually with constant trituration. To this are added slowly 5 cc. of stained oil, with constant trituration. More water is added slowly, with thorough trituration after each addition, until 100 cc. of water have been added. The emulsion is poured into a beaker. Note that the stained fat comes to the surface. (*b*) *Preparing a stable emulsion.* — Repeat (*a*) using, instead of lactose,

5 grams of powdered acacia (gum arabic), 7.5 cc. of water, and unstained oil instead of stained oil. Note the peculiar crackling sound when the oil is being emulsified into the acacia. Note the milky appearance of the diluted emulsion. Compare with the appearance of 5 grams of acacia alone, dissolved in 100 cc. of water. To what is the milky appearance of the emulsion due? Examine some of the artificial milk under the microscope. Set aside in a large test tube and note if cream rises on standing. Why is the emulsion prepared in this case so much better than the one with lactose and oil? (*c*) *A stable emulsion with milk solids.* — Repeat Part (*b*), using 8 grams of soluble skim-milk powder and 5 cc. of stained oil, diluting the emulsion to 100 cc. with water. Note that the proportions of the solids used are approximately those of whole milk. Examine the resulting milk under the microscope. Add a few drops of formalin and set aside until the next period and observe whether a cream layer has formed. (*d*) *An exceedingly fine emulsion with casein.* — Repeat Part (*b*) of this Experiment, using 5 cc. of stained oil and sufficient of the casein prepared in Experiment 9 to be equivalent to 2.5 grams of dry casein. Dissolve the casein in 20 cc. of $N/10$ NaOH. Dilute the emulsion to 100 cc. with water. Note that the proportion of casein used is approximately that of milk. Examine the resulting emulsion under the microscope. Set aside until the next period and observe whether a cream layer has formed. Examine again under microscope. What do you conclude as to the ability of sodium caseinate to hold in suspension a homogenized condition of fat?

Experiment 20. — Colloidal Dispersions

Substances in the colloidal state are not visible with the microscope but may frequently be seen with the ultramicro-

scope. Colloids also usually fail to dialyze through a suitable membrane.

The colloids of milk are calcium caseinates, $Ca_2H_2(PO_4)_2$ (and possibly some $Ca_3(P_4)_2$), most, if not all, of the lactalbumin and globulin, and probably the enzymes, which are at least adsorbed on the proteins. The appearance of a colloid particle with the ultramicroscope depends on the diffraction of a bright light by the particle when viewed against a dark background. A colloid which is strongly hydrophilic is not visible with the ultramicroscope when in a highly hydrated state because its coefficient of refraction is not very different from that of its dispersion medium. The lactalbumin and globulin are therefore not visible with the ultramicroscope. Their colloidal state will be demonstrated by the fact that only a small portion of these proteins in the milk will dialyze.

(1) *Calcium caseinate and $CaHPO_4$ in the ultramicroscope.* — Examine by the ultramicroscope fresh skim milk from which the calcium salts have been precipitated by potassium oxalate. It is not possible to differentiate between calcium caseinate and colloidal calcium salts by this means, but it is possible to show that ultramicroscopic particles are still visible after the calcium salts have been removed. The diameter of the calcium caseinate particles has been estimated at 0.13–0.17 microns. Cow's milk contains 3 to 6 billion per cubic centimeter. Further proof of the colloidal state of calcium phosphate will be furnished in the dialysis experiment (Part 3).

(2) *Rennet coagulation in the ultramicroscope.* — Watch the coagulation of skim milk with rennet in the ultramicroscope. Note that the calcium caseinate particles retain their identity after coagulation.

(3) *Dialysis of skim milk.* — For this experiment it will be necessary to prepare two collodion bags as follows:

Thoroughly clean a 300-cc. Erlenmeyer flask with chromic acid cleaning mixture and dry it or rinse with alcohol before use. Place in it 10 cc. of collodion, and then pour the excess rapidly back into the bottle, rotating the flask all the time so as to secure as uniform distribution of the collodion on the walls as possible. Continue rotating the flask for two or three minutes or until the collodion begins to dry around the mouth of the flask, or better still, until you observe the membrane on the walls of the flask taking on a *wrinkled* appearance. Then carefully fill the flask with water, and, after letting it stand for half a minute, pour out the water. Loosen the collodion around the mouth of the flask and let a few cubic centimeters of water run down between the collodion film and the walls of the flask. Rotate the flask so that the water separates all of the collodion film from the glass, draw the collodion sack up slightly, suck out the air in it so that it collapses, and then draw it out of the flask. Test it for holes by filling with water. If it contains holes, repeat. The first attempt is often a failure.

Fill one of the collodion bags with 1 per cent gelatin solution, immerse the outside of the bag in a similar solution, and let stand for fifteen minutes. Remove the bag from the gelatin solution and carefully pour out the gelatin from the interior, draining as completely as possible. Now repeat the process, using 2 per cent formaldehyde solution. Rinse the bag *carefully* and *thoroughly*, both inside and out, with cold distilled water. The bag is now ready for use.

Pour 50 cc. of fresh skim milk, with which 5 cc. of toluene have been thoroughly mixed for preservative, into each of the collodion dialyzers thus prepared, tie the top of each dialyzer together, and suspend from a glass rod into a beaker containing 300 cc. of distilled water, having the water and the milk in the dialyzer at the same level. Let stand until the next period, but not longer, preferably in the ice box.

Save the water in each beaker (the diffusate) for Experiment 21, adding 1 or 2 cc. of toluene as preservative, and placing in stoppered Erlenmeyer flasks in a refrigerator.

Combine the contents of the dialyzers and test as follows:

(*a*) Add 1 cc. of neutral, saturated potassium oxalate to 25 cc. of the fluid. Mix and let stand for about ten minutes. Is there a precipitate? What is it?

(*b*) Boil a small amount of the fluid in a beaker and note the formation of a film as in Experiment 4 (1), Chapter I.

(*c*) Acidify a small amount of the fluid. What is the precipitate?

(*d*) Test 5 cc. of the fluid for peroxidase, using the guaiac test

Experiment 21. — Molecular Dispersions

Test the diffusates containing the constituents of milk which passed through the collodion membranes as follows, and compare the results obtained with the two filters.

(1) *Proteins.* — Test for heat-coagulable material, using 10 cc. in a test tube.

(2) *Bases and peptids.* — Add 5 cc. of Almen's tannic acid reagent to 25 cc. of the diffusate.

(3) *Lactose.* — Make a Fehling's test on the diffusate.

(4) *Inorganic salts.* — (*a*) *Phosphates.* — Make 50 cc. of the diffusate faintly alkaline to phenolphthalein, using perfectly clear $Ca(OH)_2$ solution. Is there a neutralization precipitate? Test for phosphates also by acidifying with HNO_3, heating to boiling, and adding ammonium molybdate. If phosphates are present, yellow ammonium phosphomolybdate will be precipitated. (*b*) *Chlorides.* — Acidify with HNO_3 and add $AgNO_3$. (*c*) *Sulfates.* — Acidify strongly with HCl and add a few cubic centimeters of $BaCl_2$. Before interpreting results, compare with a test

in which HCl has not been added. Phosphates are precipitated by $BaCl_2$ but the barium phosphates are soluble in HCl. (*d*) *Calcium.* — Add NH_4 or K oxalate to some of the diffusate. (*e*) *Magnesium.* — See directions under Experiment 15 (5), Chapter II. (*f*) *Sodium and potassium.* — See directions under Experiment 15 (1) and (2), Chapter II.

(5) *Enzymes.* — Test the diffusates for peroxidase, using the guaiac test.

QUESTIONS

1. How do the two ultrafilters differ in structure?
2. What is the effect of this difference on the diffusion experiment with milk?
3. How can the results with the two filters be interpreted, so far as milk proteins are concerned?

CHAPTER IV

BUTTER

Experiment 22. — Structure

(1) *Normal butter.* — Place on a slide a sample of butter about the size of a pea. Cover with a cover glass and press down gently with a glass rod until the butter is spread in a very thin layer. Examine under the microscope, using a $\frac{1}{16}$ objective. Note the emulsion character of the microscopic field. The globules in the field can be identified as water by focusing on one or two drops and then gradually lowering the objective with the fine adjustment until a bright spot appears in the center of the drops before they disappear. Fat globules show this on *raising* the objective after focusing on a globule, as in milk. (Verify this.) In the dark field, water drops show a shining edge on the slide *toward* the observer but fat globules show this on the side *away* from the observer. (Verify this.)

(2) *Artificial butter.* — Warm a mortar under the hot-water tap, wipe dry, and place in a pan of hot water. Grind 1.0 gram of gum dammar to a powder in the mortar, add 5 cc. of melted butterfat, and triturate until the gum has dissolved. Now add skim milk, a few drops at a time, triturating after each addition until completely emulsified. Continue until 4 cc. of skim milk have been emulsified with the fat. Dilute the emulsion with 20 cc. of melted butterfat, triturate until it is homogeneous, then pour into a beaker of cold water which has been chilled with ice. Collect the solidified material, and press it together in a small cake with

r the microscope and compare the
ural butter, as in Part (1).
nd margarines). — A small piece
sample is placed on a glass slide,
, and examined under a polarizing
agnifications. If a selenite plate
le and the lower nicol, pure butter
m color (e.g., blue) whereas reno-
will show a colored field mottled
olors (e.g., yellow) depending on
nite plate used. Particles of salt
d the test for renovated butter
a sample of known pure butter.
ples of pure and renovated butter,
y the instructor. Rendered fats
re or renovated by this method
n the fact that, by polarized light,
rgarines) having been made from
talline state which is not present in
rendered.)

Chemical Decompositions

ty. — Add 1 gram of steapsin
per cent cream in a pint bottle.
ightly, and shake vigorously until
off the buttermilk, wash once with
side in your desk and observe the
strong rancid odor.

QUESTION

e decomposition in this experiment.

(*tallowiness*). — Add 5 cc. of dilute
½ pint of sour 25 per cent cream in

a pint bottle. Churn this into butter as in Part (1) and set aside to observe the gradual bleaching and development of strong tallowy odor. When the butter has shown the characteristic decompostion, render the fat, filter while hot, and make the Kreis test on the melted fat as in Experiment 16, (Part 11), Chapter II.

(3) *Fishy butter.* — Allow ½ pint of sweet 25 per cent cream to sour naturally in a pint milk bottle. Add 25 cc. of saturated (approximately 2.5 per cent) aqueous solution of iron lactate (ferrous lactate). Churn by shaking at the proper temperature. Drain off the buttermilk. Work sufficient salt into the butter so that it will contain about 3 per cent NaCl. Stopper and set aside in desk at same temperature. Examine from time to time for fishy odor, which should develop within four to six weeks. The conditions produced in the butter which favor fishiness are, (1) high acid, (2) high salt, and (3) iron salt as catalyst.

Experiment 24. — Approximate Analysis

(Determination of Moisture, Fat, Curd, and Salt in Single Sample of Butter by Kohman's Method, J. Ind. Eng. Chem., **11**, 36, 1919.)

(1) Weigh out a 10-gram sample of butter in a tall, 100 cc., rather narrow, lipped aluminum beaker. Drive off the moisture over a very small flame, taking the slightest noticeable browning of the sample as the end point (a slight over-browning does not affect the results appreciably). Weigh the beaker to determine moisture.

(2) Now fill the beaker with petroleum ether (or high-grade, preferably distilled or filtered gasoline), stirring the contents with a glass rod to secure a uniform mixture. Cover the beaker with a watch crystal and allow to stand for three to five minutes for the mixture of curd and salt to settle out. The solvent is then gently decanted off without

disturbing the sediment. The beaker is then filled with fresh solvent. The curd and salt mixture settles rapidly in the fresh solvent and the liquid can be decanted off in a very short time.

(3). Heat the beaker gently, either on a water bath, a hot plate, or directly over a small alcohol flame, but not so rapidly as to cause sputtering. The tendency to sputter can be lessened by tapping the beaker on the table before trying to evaporate off the solvent. When the sediment is free from solvent, the beaker is weighed again. The difference between the moisture-free weight and this weight, multiplied by 10, gives the percentage of fat in the butter.

(4) Now fill the beaker half full of hot distilled water and let stand for a few minutes with occasional stirring to insure the solution of the salt. Add a few drops of chromate indicator and add, from a burette, silver nitrate solution of such strength that 10 cc. equals $\frac{1}{10}$ gram of salt. (Such a solution contains 29.062 grams silver nitrate to 1000 cc. of distilled water.) The number of cubic centimeters of silver nitrate required to give a permanent brown color, divided by 10, will give the percentage of salt in the butter.

(5) The percentage of curd in the butter can be calculated by adding together the percentages of moisture, fat, and salt, and subtracting from 100.

CHAPTER V

RENNET COAGULATION

In the various rennet studies, the proportion of rennet extract added is greatly in excess of the minimum amount required, probably twenty times too much being added. This has no bearing on the results except to accelerate the speed of the coagulation. *Great care should be taken, therefore, in experiments in which time of coagulation is one of the factors being studied, to make all conditions as nearly alike as possible, especially as to the amount of rennet extract added in comparative tests.* A pan of water on a tripod with a small flame under it serves as a very satisfactory thermostat for these experiments if the temperature of the water is watched closely.

Experiment 25. — Influence of Temperature

(1) *Temperature of milk.* — Prepare three beakers containing 200 cc. each of fresh milk and compare the rate at which 1 cc. of diluted rennin solution* coagulates the milk at (*a*) 5–10° C., (*b*) 40° C., and (*c*) 55° C., respectively, maintaining the milk at the required temperature for at least one hour. Bring the milk to the required temperature before adding the rennet, and distribute the rennet in

* Diluted rennin solution is prepared by diluting 5 cc. of fresh rennet extract to 50 cc. with distilled water. The diluted rennin solution should be of such strength that 1 cc. will clot 100 cc. of fresh milk in about ten minutes at 40° C. A few preliminary tests by the instructor will determine the correct dilution for the rennet extract at hand and will help insure the success of the rennet coagulation experiments.

the fluid as quickly and thoroughly as possible by means of a stirring rod.

(2) *Effect of heat on rennin.* — Heat some diluted rennin solution to 60° C. for thirty minutes. Compare the rate of coagulation of 100-cc. portions of milk at 40° C. when 1 cc. of heated, and when 1 cc. of unheated rennin solution is added.

Experiment 26. — Influence of Previous Heating of Milk and Relation thereto of Added Bivalent Salts

Prepare eight beakers each containing 160 cc. of fresh milk. Keep Beakers 1 and 2 at room temperature. Heat Beakers 3 and 4 to 60–65° C. for thirty minutes in a water bath. Heat Beakers 5 and 6 to 75° C. for five minutes in a hot water bath, being sure that the milk reaches the prescribed temperature. Heat Beakers 7 and 8 to boiling and boil gently for two minutes.

Now bring the temperature of all the beakers to 40° C. and place in thermostat. Add 1.5 cc. of $M/2$ $CaCl_2$ solution to Beakers 2, 4, 6, and 8, mixing the calcium solution thoroughly, and compare as nearly as possible the time of coagulation among the different beakers, particularly between the beakers of each pair, to one of which $CaCl_2$ was added. Also compare the character of the curd in the several tests (firm, soft, flaky, etc.).

QUESTION

How would you interpret the results of these tests?

Experiment 27. — Influence of Reaction of Milk

Prepare five beakers each containing 100 cc. of milk. Add sufficient 5 per cent Na_2CO_3 solution to Beaker 2 so that the milk contains exactly 0.5 per cent Na_2CO_3. Add

sufficient 10 per cent lactic acid to Beaker 3 so that theoretically the milk contains 0.3 per cent lactic acid. Add lactic acid to Beaker 4 so that theoretically the milk contains 0.05 per cent lactic acid. Add 2.5 cc. of 5 per cent NaH_2PO_4 solution to Beaker 5. The lactic acid should be added cautiously, a little at a time, with rapid stirring.

Bring the temperature of each beaker to 40° C., place in the thermostat, and add 1 cc. of diluted rennin solution to each beaker. Note time of coagulation and compare results. Give an explanation for each result. Also note character of the curd.

Experiment 28. — Influence of Bivalent Cations

Prepare six beakers, each containing 100 cc. of fresh skim milk. Add 0.5 cc. saturated potassium oxalate ($pH = 6.5$) to each beaker, and let stand for five minutes. Warm all samples to 40° C. in the water bath and keep them at that temperature.

Beaker 1. Add 1 cc. diluted rennin solution (1 cc. extract in 10 cc. water).

Beaker 2. Add 2 cc. $M/2$ $CaCl_2$ by placing the $CaCl_2$ in a clean beaker and pouring the warm oxalated milk into the $CaCl_2$ and mixing carefully by pouring. Now add 1 cc. diluted rennin solution.

Beaker 3. Add 20 cc. of clear lime water, mix, and add 1 cc. diluted rennin.

Beaker 4. Add 2 cc. of $M/2$ $BaCl_2$, using method as for Beaker 2. Now add 1 cc. rennin solution.

Beaker 5. Add 2 cc. $M/2$ $MgCl_2$ and 1 cc. rennin as for Beakers 2 and 4.

Beaker 6. Add 2 cc. $M/2$ $ZnCl_2$ and 1 cc. rennin as in other tests.

Note time of clotting and character of clot in each test.

Allow ten or fifteen minutes before deciding that no clotting will occur.

Explain the results with the different bivalent cations.

Experiment 29. — Influence of Dilution

(1) *Diluting the rennin.* — Prepare three beakers each containing 100 cc. of fresh skim milk. Warm to 40° C. in water bath and add 1 cc. of *undiluted* rennet extract to Beaker 1, 1 cc. of 10 per cent rennet extract to Beaker 2, and 1 cc. of 1 per cent rennet extract to Beaker 3. Compare the rate of clotting and character of clot.

(2) *Diluting the milk.* — Prepare four beakers containing respectively 100 cc., 90 cc., 75 cc., and 50 cc. of fresh milk. Dilute Beakers 2, 3, and 4, to 100-cc. volume with distilled water. Warm the set to 40° C. and add 1 cc. of diluted rennin solution. Compare the rate of coagulation and character of clot.

Experiment 30. — Influence of Added Colloids

(1) *Gelatin.* — Add 10 cc. of freshly prepared 10 per cent solution of powdered gelatin to 100 cc. of fresh milk and compare the rate of coagulation and character of clot of this milk and untreated milk to which 10 cc. of water have been added, on addition of 1 cc. of diluted rennin solution at 40° C.

(2) *Gum acacia.* — Add 10 cc. of freshly prepared 10 per cent solution of powdered gum acacia to 100 cc. of fresh milk, and study its coagulation with rennet as in Part (1).

CHAPTER VI

QUALITATIVE ANALYSIS OF CHEDDAR CHEESE

Experiment 31. — Water-soluble Proteins in Cheddar Cheese

The bulk of the proteins of ripened cheddar cheese are dispersable in water. Among them there usually can be identified (*a*) paranuclein, insoluble in dilute HCl; (*b*) sometimes protein, which is heat-coagulable when in neutral solution, especially in cheese ripened at a very low temperature; (*c*) caseoses, precipitated from acid solutions on saturation with $ZnSO_4$; (*d*) peptones, not precipitated by $ZnSO_4$, but precipitated from caseose-free solutions by tannic acid or by phosphotungstic acid; (*e*) amino acids, not precipitated by the above reagents, except for certain of the amino acids; (*f*) ammonia.

Thoroughly grind 50 grams of cheddar cheese in small portions in a mortar, with an equal bulk of clean quartz sand. Transfer to a 300-cc. Erlenmeyer flask and add 200 cc. of distilled water at 50° C. Keep the mixture at this temperature for about thirty minutes, shaking vigorously from time to time. Then decant the liquid portion through a filter of absorbent cotton prepared as stated below. Repeat the extraction with a second 200-cc. portion of distilled water, decanting through the same filter and saving the combined filtrates. Centrifuge the combined filtrates for fifteen minutes and pipette out the clearest middle layer, free from supernatant fat and insoluble particles, for Parts (1), (2), (3).

The cotton filter is prepared as follows: In a 6-inch

glass funnel place some absorbent cotton to a depth of about 1 inch, moisten this with water, in order to compact it, and then above this place a second layer of cotton of the same thickness. Upon this pour the portions of cheese extract. Do not pack the cotton down too tightly.

After the second extraction return the upper layer of cotton containing the solid particles to the extraction flask for extraction with salt solution. (See Experiment 33.) The extracts are to be used for remainder of this experiment and for Experiment 32.

(1) *Paranuclein.* — To 100 cc. of water extract, add 5 cc. of 1 per cent solution of HCl, and warm the mixture to 50° C. until a complete separation takes place. Filter off the precipitate, dissolve in dilute NaOH, and test for protein with biuret reaction. Save the filtrate for (2)

Paranuclein results from the breaking down ot paracasein by the cheese-ripening enzymes, and is always found in the water extracts of cheddar and many other kinds of cheese.

(2) *Heat-coagulable protein.* — Neutralize the filtrate from the paranuclein separation with dilute alkali, using phenolphthalein as indicator. Add 10 per cent acetic acid to give a concentration of 0.5 per cent. Heat to boiling and continue until positive or negative evidence of heat-coagulable material is obtained

(3) *Caseoses.* — If heat-coagulable material is obtained in (2) filter it off and proceed as follows with the filtrate. If no heat-coagulable material is obtained, proceed with the solution.

Let filtrate (or solution) cool slightly, i.e., to about 70° C., add 1 cc. of 50 per cent H_2SO_4, and saturate with $ZnSO_4$ crystals at that temperature (100 cc. water dissolve 90 grams of $ZnSO_4$ at 70° C). Dissolve a portion of the precipitate in water and test with biuret reaction. Use strong NaOH and warm to destroy phenolphthalein color before

making biuret test. Note the pink color obtained, which is characteristic of proteoses.

(4) *Ammonia.* — Place a fresh 50-cc. portion of water extract of cheese in an Erlenmeyer or Florence flask of 200-cc. capacity, add about *25 grams of MgO*, and heat to boiling. Test the vapors for ammonia by odor and with red litmus paper.

(5) and (6) *Peptones and amino compounds.* — (Notes for information only.) Peptones and amino compounds are difficult to detect directly. Their presence in cheese is determined indirectly in quantitative work in the following way. The proteoses and peptones are precipitated together by one of the alkaloidal reagents, such as Almen's tannic acid reagent, or phosphotungstic acid, and the nitrogen determined as caseoses in (3) subtracted from the total precipitated as caseoses and peptones.

The filtrate from the caseoses and peptones contains nitrogen as amino compounds and ammonia. By determining the total nitrogen and the ammonia nitrogen in the filtrate, and finding the difference between them, the amino nitrogen may be determined.

Experiment 32. — Lactose and Lactic Acid in Water Extract of Cheese

(1) *Lactose.* — Test a portion of the water extract obtained in Experiment 31 for sugar, by Fehling's test.

(2) *Lactic acid.* — Concentrate 50 cc. of water extract of cheese to about 5 cc. on the water bath, using a small porcelain dish, and add (cold) to 5 cc. of Uffelmann's reagent in a test tube. A canary-yellow or greenish-yellow color develops in the presence of 0.01 per cent lactic acid or over. The reagent is prepared by adding dilute ferric chloride solution to 1 per cent carbolic acid until an amethyst-blue color is obtained.

Experiment 33. — "Brine-soluble" Protein of Cheese

In the manufacture of cheddar cheese the calcium caseinates of the milk are first changed to calcium paracaseinates by the action of the rennet enzyme. In the presence of lactic acid these calcium salts of paracasein are rapidly changed in the cheddaring process to more acid calcium paracaseinates. During ripening these give way gradually to insoluble paracasein which decomposes into paranuclein and other less complex soluble compounds. The calcium paracaseinates never completely disappear from cheddar cheese, even after many months of ripening. The calcium paracaseinate, which is soluble in dilute NaCl solution, has been called mono-calcium paracaseinate, but this is based on the assumption that paracasein has a molecular weight of 4,444, which is improbable.

Dissolve 10 grams of NaCl in 200 cc. of water and add the solution to the water-insoluble material of the cheese extracted with water in Experiment 31. Digest at 50° C. for one hour, with frequent shaking, and filter through an absorbent cotton filter as in Experiment 31. Cool the filtrate to about 40° C. and add 10 per cent acetic acid, a little at a time, with stirring, until a clear coagulation occurs. How does this experiment resemble the precipitation of casein from milk? Tabulate the results obtained in Experiments 31, 32, and 33.

CHAPTER VII

QUALITATIVE EXAMINATION OF MILK POWDER

Milk powder, especially skim-milk powder, is used widely in creameries for the making of starter, and in ice-cream manufacture to make artificial cream. Only the highest-grade powder should be used, since the method used for making the powder may result in a very inferior product, which will not give a natural milk when redissolved. The following experiments, when applied to a sample of powder, will show whether the method of manufacture has injured any of the constituents of the milk.

Experiment 34. — Examination of a High-grade Skim-milk Powder

Dissolve 25 grams of the powder in 200 cc. of distilled water. Reserve 150 cc. of the resulting milk for the rennet and peroxidase tests (see below). Dilute the remainder to 200 cc. Heat to 40° C. and add 10 per cent acetic acid, a few drops at a time, with stirring, until a clear coagulation of the casein results. Filter off the casein. Add a few drops of phenolphthalein indicator to the filtrate and carefully neutralize with lime water. Is there a neutralization precipitate?

Add 0.5 cc. of 10 per cent acetic acid to the solution and heat to boiling. Is there a coagulation of proteins? Boil for about fifteen minutes and filter off the heat-coagulable proteins if any form. Make a Fehling's test on a portion of the filtrate. Is lactose present? Add an equal volume

of Almen's tannic acid reagent to 25 cc. of the filtrate. Is there any further precipitation of protein?

Rennet tests. — Divide 140 cc. of the milk, reserved from above, into two parts. Add 2 or 3 drops of 1 per cent lactic acid to each part and add 0.5 cc. of $M/2$ $CaCl_2$ solution to one part, but not to the other. Warm both parts to 38° C. in a large vessel of warm water, and add 1 cc. of diluted rennin solution to each part. Note the time required for the milk to clot in each. Is there any difference?

Peroxidase. — Test 5 cc. of the milk for peroxidase, using the guaiac and Storch tests.

Compare the results of all of the foregoing tests with the results obtained in similar tests which you made on normal milk in some of the previous exercises.

This exercise is optional.

CHAPTER VIII

QUANTITATIVE ANALYSIS OF MILK

Experiment 35. — Moisture, Total Solids, and Fat

(1) *Moisture and total solids (standard method).* — Determine the specific gravity of the milk with Westphal balance (or accurate lactometer) and very carefully and accurately measure out 2 cc. of the milk, at the temperature at which the sp. gr. was taken, into a previously dried and weighed, flat metal dish containing a thin layer of pure quartz sand. Dry at the temperature of boiling water until the dish (plus milk) ceases to lose weight. Dry for two hours, cool and weigh, and then dry for half-hour periods; cool in a desiccator after each heating and weigh rapidly to avoid absorption of hygroscopic moisture. Calculate the percentage of moisture and total solids in the milk, taking into account the sp. gr. and the volume of milk taken.

(2) *Fat* (Röse-Gottlieb method). — Measure 10 cc. of milk (sp. gr. of which has been determined), with accurate pipette, into a Röhrig tube or some similar apparatus, add 1.25 cc. of concentrated NH_4OH (2 cc. if sample is sour), and mix thoroughly with the milk. Add 10 cc. of 95 per cent alcohol and mix well. Add 25 cc. of washed ethyl ether and shake vigorously for half a minute; then add 25 cc. of petroleum ether (preferably distilling under 60° C.), and shake again for half a minute. *Let stand for twenty minutes,* or until the upper liquid is practically clear and its lower level constant. Remove as much as possible of the ether-fat solution (usually 0.5 to 0.8 cc. will be left) into a previously weighed flask through a small quick-acting

filter of selected paper. The flask should always be weighed with a similar one as a counterpoise to compensate for variations in weight of glass flask. Re-extract the liquid remaining in the tube, this time with only 15 cc. of each ether, shaking vigorously for half a minute with each, and allow to settle. Remove the clear solution through the small filter into the same flask as before, and wash the tip of the spigot of the Röhrig tube, the funnel, and the filter with a few cubic centimeters of a mixture of the two ethers in equal parts (previously mixed and free from deposited water). Evaporate the ether slowly on a hot-water or steam bath, then dry at temperature of boiling water for half-hour periods until there is no further loss in weight, cooling in desiccator after each weighing, and weighing rapidly.

Experiment 36. — Casein (Volumetric Method of Van Slyke and Bosworth)

(Slightly Modified from Geneva, N. Y., Agr. Expt. Sta. Tech. Bul. 10, 1909)

Apparatus required. — Two 50-cc. burettes, two 200-cc. graduated volumetric flasks, two rapid-filtering funnels, one 17.5-cc. milk pipette, one 100-cc. pipette, two 250-cc. beakers, one 300-cc. Erlenmeyer flask, color comparator box for determining isoelectric point of casein, six test tubes for box.

Solutions required. — Standard sodium hydrate and hydrochloric acid solutions, 1 per cent alcoholic phenolphthalein, 0.04 per cent alcoholic methyl red solution.

Theory of method. — This method is based on principles which conform to our present knowledge of the chemistry of milk.

Casein forms a definite compound with bases at a pH corresponding to neutrality to phenolphthalein; the casein

acts as an acid and neutralizes between 8 and 9 cc. of 0.1 *N* NaOH per gram of casein. Van Slyke and Bosworth, who devised the method, assumed a somewhat arbitrary value of 8.84 cc. of 0.1 *N* NaOH equivalent to 1 gram of casein (1 cc. 0.1 *N* NaOH = 0.1131 gram of casein). This value can be accepted as sufficiently close, inasmuch as a slight experimental error is involved in selecting the end point of titration with phenolphthalein.

In carrying out the method, alkali is first added, in order to convert the casein into the compound neutral to phenolphthalein, as well as to neutralize the various buffer salts to the same *pH*. The alkali caseinate is now changed to base-free casein by adding acid until a *pH* of 4.6, the isoelectric point of casein, is reached. The base-free, isoelectric casein is filtered off and the filtrate again neutralized to the *pH* of the alkali-caseinate as represented by neutrality to phenolphthalein. The difference between the acid required to bring the milk to a *pH* of 4.6 and the alkali required to bring the casein-free filtrate back to the *pH* at which the original sample of milk was neutralized, represents the acid corresponding to the casein removed. The accuracy of the method depends largely on the selection of the end points of the titrations. Inasmuch as the end points of the titrations with alkali are not difficult, the largest error is introduced in the titration with acid to the isoelectric point of casein. The original method does not call for any control of this end point. The method given here is modified to include such control; the addition of the methyl red indicator does not interfere with the subsequent end point with phenolphthalein used as indicator. The method is modified also in the use of a stronger acid in place of acetic acid, which is readily adsorbed by the casein.

Method. — By using NaOH of such a strength that 1 cc. corresponds to 0.09 grams of casein, and HCl of the same

strength as the NaOH, the milk can be measured with a 17.6-cc. pipette (18 grams) if the proper volumes are maintained, and the percentage of casein can be determined directly from the alkali and acid used.

The standard alkali is prepared by diluting 795 cc. of 0.1 *N* NaOH to 1 liter, and the standard HCl in the same manner.

Perform the following test in *duplicate.*

Measure 17.6 cc. of milk into a 200-cc. graduated flask. Add 1 cc. of 1 per cent phenolphthalein solution. Add 80 cc. of water at room temperature and run in the standard alkali from a burette until a *faint permanent* pink color prevails. The alkali should be added at the rate of about 1 drop per second. Excess of alkali *must* be avoided. Now add standard HCl until a pH of 4.6 is reached, using the following procedure. First add slowly, from a burette, about 12 cc. of the acid, at the rate of about 1 drop per second. If the casein does not settle fairly sharply from the surrounding fluid, continue the addition of acid in 0.2-cc. portions until this occurs. Now add 1 cc. of methyl red indicator. Mix thoroughly. Carefully pour out approximately 10 cc. of the supernatant fluid into a test tube on which a 10-cc. mark has been placed. Match the color with the indicator standard $pH = 4.6$, used in Experiment 9, Chapter II. Each 10 cc. of standard buffer should contain 0.08 cc. of methyl red indicator. Add 2 more drops of methyl red indicator to the unknown.* If the fluid does

* IMPORTANT NOTE: — In order to make this color comparison as accurate as possible, the comparator box should have places for six test tubes in two rows of three each. Place the standard buffer tubes in the two outer places of the front row, and a tube with distilled water in the center. Place the unknown behind the distilled water. Flanking the unknown, behind the standard buffers, are tubes containing supernatant fluid without any methyl red, from a separate casein precipitation test prepared as follows: Measure into a beaker 17.6 cc. of the milk being analyzed. Add 80 cc. of distilled water. Add standard acid, slowly, with stirring, until the casein breaks sharply as in the quantitative test. Allow the casein to settle and use the supernatant

not show that a pH of 4.6 has been reached, return the fluid to the flask and continue the addition of HCl in 0.2-cc. portions until this pH is reached, testing the pH of the supernatant fluid after each addition of acid. Two drops of indicator must be added to each portion tested, because the casein adsorbs considerable of the methyl red. Remember to return the fluid to the flask after each test. Before making the final color comparison, shake the flask for one minute with a strong rotary motion. When the proper pH has been reached, rinse the test tube into the flask. *Record the amount of HCl used as A.*

Fill the flask exactly to the 200-cc. mark with distilled water, shake vigorously for half a minute to insure a uniform distribution of acid, and filter off the casein on a dry filter, catching the filtrate in a dry beaker. The filtrate should be perfectly clear, or should show only a faint opalescence, due to fat globules, if the milk was rich in fat.

When most of the filtrate has come through, pipette out 100 cc. into an Erlenmeyer flask and titrate through the bright yellow color of the alkaline methyl red to a faint permanent orange with the standard NaOH solution (no additional phenolphthalein needed). *Record the amount of alkali used as B.*

The percentage of casein in the milk will be $\frac{A}{2} - B$.

Experiment 37. — Determination of Added Water in Milk

(Using the Hortvet Cryoscope)
(Directions from Bul. 268, Eimer and Amend Company)

(1) *Determination of the freezing point of water.* — Insert a small-caliber thistle-tube or funnel-tube into the vertical

fluid for the test tubes to be placed behind the standard buffers. These will serve for the entire experiment and need not be poured out until satisfactory results have been secured for the analysis.

portion of the T-tube at one side of the apparatus and pour in about 400 cc. of ether, previously cooled to 15° C. or lower. Close the vertical tube by means of a small cork and connect the pressure bulb or pump to the air-inlet tube of the air-drying attachment on the opposite side of the apparatus.

Measure into the inner test tube 30–35 cc. of boiled distilled water, previously cooled to 10° C. or lower. Enough water should be measured in fairly to submerge the thermometer bulb. Insert the thermometer, which, together with the stirring device, is mounted in a second stopper, and lower the test tube, which is tightly fitted into the apparatus. A small quantity of alcohol, sufficient to fill the space between the two test tubes, will serve to complete the conducting medium between the interior of the apparatus and the liquid to be tested. A sufficiently tight connection between the inner and outer tubes is afforded by means of a narrow section of thin-walled tubing. The thermometer and stirring device should fit accurately in the stopper and the entire arrangement should be in a vertical position. By means of the pressure pump, maintain a steady current of air through the apparatus, thereby vaporizing the ether at a fairly rapid rate. Arrangement may be made to conduct the ether vapors away from the operator, and the *apparatus should not be used in the vicinity of a flame.* Keep the stirring device in steady up-and-down motion at a rate of about one stroke each two or three seconds, or even at a slower rate, provided the cooling proceeds satisfactorily. Maintain passage of air through the apparatus until the temperature of the ether cooling bath approaches 3 degrees below zero, as indicated on the control thermometer, or until the top of the mercury thread in the special freezing-point thermometer recedes to a point in the neighborhood of the probable freezing point of water. Continue the manipu-

lation of the stirring device until a super-cooling of from 1.2 to 1.3 degrees is observed. Also note the temperature recorded on the control thermometer, in order to guard against excessive super-cooling and a consequent too low convergence temperature. As a rule, by this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury thread. Manipulate the stirring device slowly and carefully two or three times while the mercury column approaches its highest point. By means of a suitable light-weight mallet, tap the upper end of the thermometer cautiously several times in order to insure a permanent position of the top of the mercury column. Observe the exact reading on the thermometer scale and estimate to 0.001 degree C. After a few minutes' time the mercury may begin to recede, owing to the cooling effect of the ether in the interior of the apparatus. When the above observation has been satisfactorily completed, make a couple of duplicate determinations, then remove the thermometer and stirring device and empty the water from the inner tube.

(2) *Determination of the freezing point of normal milk.* — Rinse out the test tube with about 20 cc. of fresh milk, measure into the tube about 35 cc. of the milk, or enough fairly to submerge the thermometer bulb, and insert the tube into the apparatus. In the meantime, by lowering a narrow tube into the ether bath, then closing the top end by means of the forefinger and raising to a suitable height, it will be possible to ascertain whether an additional supply of ether is necessary for the determination. Usually an additional 50 to 75 cc. of cold ether should be poured in at this stage. When the apparatus has been cooled down to a low temperature, an additional 50 cc. of ether is sufficient on an average for four or five additional determinations. Make the determination of the sample of milk, following

the same procedure as that employed in determining the freezing point of water. As a rule, however, it is necessary to start the freezing action in the sample of milk by dropping in a small fragment of ice (approximately 0.05 gram) at the time when the mercury column has receded to a place from 1.2 to 1.3 degrees below the probable freezing point. A rapid rise of the mercury column results almost immediately. Manipulate the stirring device slowly and carefully two or three times while the mercury column approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the determination of the freezing point of water, then observe the exact reading on the thermometer scale and estimate to 0.001 degree. The algebraic difference between the reading obtained on the sample of water and the reading obtained on the sample of milk represents the freezing point of the milk.

(3) *Determining the freezing point of watered milk.* — Repeat Part (2) on samples of milk to which 5 and 10 per cent water, respectively, have been added. Calculate the amount of added water from the freezing point, by using the porcelain scale on the cryoscope and also from the formula:

$$W = 100 \times \frac{(T - T') - (T - T'')}{T - T'}$$

where W = the added water
T = observed freezing point of water
T' = observed freezing point of normal milk
T'' = observed freezing point of watered milk.

REFERENCES

The following is a list of selected references for supplementary reading in connection with the various phases of the laboratory work. The references are grouped together to indicate the experiments upon which they have a bearing.

CHAPTER I

Experiments 1–3

1. The specific gravity of milk. Richmond. Dairy Chemistry, 3rd Edition, Chap. V, pp. 66–81. Chas. Griffin Co. Ltd., London, 1920.

2. The estimation of total solids in milk by the use of formulas. R. H. Shaw and C. H. Eckles. U. S. D. A. Bul. 134, 1911.

3. Relation of solids in milk to fat and specific gravity. O. R. Overman, F. A. Davidson, and F. P. Sanmann. Ill. Agr. Exp. Sta. Bul. 263, 263–271, 1925.

Experiments 4–5

1. Changes in milk produced by the action of heat and acids. J. H. Kastle and N. Roberts. U. S. Hyg. Lab. Bul. 56, Milk and its Relation to the Public Health, 328–332, 1909.

2. Changes occurring in milk as a result of the application of heat. Janet E. Lane-Claypon. Milk and its Hygienic Relations, pp. 225–243. Longmans, Green and Company, London, 1916.

3. The composition of milk membranes. W. Friese. Milchwirtschaft. Forsch., **1**, 316–325, 1925.

4. The heat coagulation of milk. H. H. Sommer and E. B. Hart. J. Biol. Chem., **40**, 137–151, 1919.

5. A preliminary report on the study of the temperature at which milk of different percentage of acidity will coagulate. T. J. McInerney. J. Dairy Sci., **3**, 220–226, 1920.

6. The relation of acidity to the coagulation temperature of evaporated milk. L. A. Rogers, E. F. Deysher, and F. R. Evans. J. Dairy Sci., **4**, 294–309, 1921.

7. The heat coagulation of milk. H. H. Sommer and E. B. Hart. J. Dairy Sci., **5**, 525–543, 1922.

8. Factors influencing the heat coagulation of milk, and the thickening of condensed milk. Alan Leighton and E. F. Deysher. Proc. World's Dairy Congress. I, 1276–1284, 1923.

9. The heat coagulation of milk. H. H. Sommer. Proc. World's Dairy Congress. I, 1241–1248, 1923.

10. The alcohol test as a means of determining quality of milk for condenseries. A. O. Dahlberg and H. S. Garner. U. S. D. A. Bul. 944, 1–13, 1921.

11. The alcohol test as a means of detecting abnormal milk. A. C. Weimar. J. Dairy Sci., **6**, 95–101, 1923.

Experiments 6–7

1. The viscosity and surface tension of milk. B. Kobler. Arch. Physiol. (Pflüger's) **125**, 1–72, 1908.

2. The viscosity of cream. F. M. Dumaresq. Proc. Roy. Soc. Victoria, **25** (N.S.), Part 2, 307–322, 1913.

3. Viscosity, surface tension, and whipping properties of milk and cream. A. C. Dahlberg and J. C. Hening. N. Y. Agr. Exp. Sta. Tech. Bul., 113, 1–42, 1925.

Experiment 8

1. The cause of acidity of fresh cow's milk. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **19**, 73–76, 1914.

2. Conditions causing variations in the reaction of freshly drawn milk. L. L. Van Slyke and J. C. Baker. J. Biol. Chem., **40**, 345–355, 1919.

3. The hydrogen-ion concentration of milk. J. Tillmans and W. Obermeier. Z. Nahr. Genussm., **40**, 23–34, 1920.

4. The relation of the natural acidity in milk to composition and physical properties. F. E. Rice and A. L. Markley. J. Dairy Sci., **7**, 468–483, 1924.

5. The Determination of Hydrogen Ions. W. M. Clark, 2nd Edition. Williams and Wilkins Co., Baltimore, 1922.

CHAPTER II

Experiment 9

1. Studies on technical casein. II. Grain-curd casein. W. M. Clark, H. F. Zoller, A. O. Dahlberg, and A. C. Weimar. J. Ind. Eng. Chem., **12**, 1163–1165, 1920.

2. Precipitation of grain-curd casein from pasteurized milk, including sweet cream buttermilk. H. F. Zoller. J. Ind. Eng. Chem., **13**, 510, 1921.

3. The preparation of pure casein. L. L. Van Slyke and J. C. Baker. J. Biol. Chem., **35**, 127–136, 1918.

4. The chemistry of casein. L. L. Van Slyke. Proc. World's Dairy Congress, II, 1145–1151, 1923.

Experiment 10

1. Composition and properties of some casein and paracasein compounds and their relation to cheese. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **14**, 211–237, 1913.

2. Condition of casein and salts in milk. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **20**, 135–154, 1915.

3. Changes in the physical condition of colloids. XXII. The general chemistry of the caseinates. W. Pauli and J. Matula. Biochem. Z., **99**, 219–236, 1919.

4. Casein, its preparation and technical utilization. 3rd Edition. Robert Sherer. Scott, Greenwood and Son, London, 1921.

5. The influence of the method of manufacture on the use of casein in glue making. Samuel Butterman. J. Ind. Eng. Chem., **12**, 141–144, 1920.

6. Casein and its industrial applications. Special report. Natural Resources Intelligence Service, Dept. of Interior, Ottawa, Canada, 1925.

Experiment 11

1. Contribution to the study of milk proteins. John Sebelein. Z. physiol. Chem., **9**, 445–464, 1889.

2. The composition of colostrum, especially its proteins. H. Tiemann. Z. physiol. Chem., **25**, 363–392, 1898.

3. A comparative study of the proteins of colostrum and milk of the cow and their relations to serum proteins. C. Crowther and H. Raistrick. Biochem. J., **10**, 434–452, 1916.

4. The proteins of cow's milk. T. B. Osborne and A. J. Wakeman. J. Biol. Chem., **33**, 7–17, 1918.

5. The physico-chemical state of the proteins in cow's milk. L. S. Palmer and R. G. Scott. J. Biol. Chem., **37**, 271–284, 1919.

6. The serum proteins of milk. W. Grimmer, C. Kurtenacker and R. Berg. Biochem. Z., **137**, 465–483, 1923.

7. Significance of temperature coefficient for the study of milk pasteurization. O. Rahn. Milchwirtschaft. Forsch., **2**, 373–382, 1925.

Experiment 12

1. Some new constituents of milk. III. A new protein, soluble in alcohol. T. B. Osborne and A. J. Wakeman. J. Biol. Chem., **33**, 243–251, 1918.

Experiment 13

1. On the mode of production of lactose in the mammary gland. D. N. Paton and E. P. Cathcart. J. Physiol., **42**, 179–188, 1911.

2. Lactose and its behavior in aqueous solutions. W. Fleischmann and G. Wiegner. J. Landw., **58**, 45–64, 1910.

3. The utilization of whey. III. The extraction of lactose and lactalbumin from fresh and condensed whey. L. Harding. J. Roy. Agr. Soc. England, **83**, 78–96, 1922.

4. Sandy crystals in ice cream: Their separation and identification. H. F. Zoller and O. E. Williams. J. Agr. Res., **21**, 791–795, 1921.

5. Factors influencing the crystallization of lactose. Alan Leighton and P. N. Peter. Proc. World's Dairy Congress, I, 477–485, 1923.

6. Sandy ice cream. C. Dahle. Proc. World's Dairy Congress, I, 500–510, 1923.

7. Lactose. O. E. Whittier. Chemical Reviews, **2**, 85–125, 1925.

Experiment 14

1. Chemical changes in the souring of milk. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **24**, 191–202, 1916.

2. Free lactic acid in sour milk. L. L. Van Slyke and J. C. Baker. J. Biol. Chem., **35**, 147–178, 1918.

3. Relation between lactic acid production and bacterial growth in the souring of milk. J. C. Baker, J. D. Drew, and H. J. Conn. N. Y. Agr. Exp. Sta. Tech. Bul. 74, 1–24, 1919.

Experiment 15

1. The salts of milk and their relation to the state of the casein. F. Söldner. Landw. Vers.-Sta., **35**, 351–436, 1888.

2. Lime and other mineral constituents in cow's milk. A. Trunz. Z. physiol. Chem., **140**, 263–310, 1903.

3. Condition of casein and salts in milk. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **20**, 135–154, 1915.

4. The distribution of saline substances and mineral elements in milk. Chas. Porcher and A. Chevallier. Compt. rend., **172**, 1605–1607, 1921.

5. The distribution of milk salts and their chemical and physical relation to the other constituents of milk. Chas. Porcher and A. Chevallier. Le lait, **3**, 97–112, 188–200, 1923.

Experiment 16

1. A contribution to the chemistry of butterfat. C. A. Brown. Ann. Rep. Penn. State College, pp. 207–245, 1899–1900.

2. Improved methods for fat analysis. E. B. Holland, J. C. Reed, and J. P. Buckley. Mass. Agr. Exp. Sta. Bul. 166, 91–138, 1915.

3. Determination of fatty acids in butterfat. I, E. B. Holland and J. P. Buckley. J. Agr. Res., **12**, 719–732, 1918; II, E. B.

Holland, M. E. Garvery, H. B. Pierce, A. C. Messer, J. G. Archibald, and C. O. Dunbar. J. Agr. Res., **24**, 365–398, 1923.

4. The analysis of butterfat. Richmond. Dairy Chemistry. 3rd Edition, 240–268. Chas. Griffin & Co. Ltd., London, 1920.

5. Glycerides of butterfat. C. Amberger. Z. Nahr. Genussm., **26**, 65–85, 1913; II, ibid. **35**, 313–381, 1918.

6. The fatty acids of butter. Ida Smedley. Biochem. J., **6**, 451–461, 1912.

7. Compounds developed in rancid fats, with observations on the mechanism of their formation. W. C. Powick. J. Agr. Res., **26**, 323–362, 1923.

Experiment 17

1. Properties of galactose, a digestive ferment of milk. S. M. Babcock, H. L. Russell, and A. Vivian. 15th Ann. Rep. Wis. Agr. Exp. Sta., pp. 82–92, 1898.

2. Chloroform as an aid in the study of milk enzymes. H. A. Harding and L. L. Van Slyke. N. Y. Agr. Exp. Sta. Tech. Bul. 7, 41–82, 1907.

3. Effect of heat on milk enzymes. J. H. Kastle and N. Roberts. U. S. Hyg. Lab. Bul. 56, 332–338, 1909.

4. The biological properties of milk, both of the human species and of cows, considered in special relation to the feeding of infants. Janet E. Lane-Claypon. Report to the Local Government Board (N.S.), No. 76, 1–95, 1913.

5. The so-called biological properties of milk. A. The ferments or enzymes. Janet E. Lane-Claypon. Milk and its Hygienic Relations, pp. 63–107. Longmans, Green & Co., London, 1916.

6. The ferments of the mammary gland and of milk. W. Grimmer. Biochem. Z., **53**, 429–473, 1913.

7. Is lipase a normal constituent of cow's milk? L. S. Palmer. J. Dairy Sci., **5**, 51–63, 1922.

8. Bitter milk of advanced lactation. A lipase fermentation. L. S. Palmer. J. Dairy Sci., **5**, 201–211, 1922.

9. Quantitative method for determination of peroxidase in

milk. F. E. Rice and T. Hanzawa. J. Ind. Eng. Chem., **14**, 201–203, 1922.

10. The presence of amylase in milk and cheese. M. Sato. Biochem. J., **14**, 120–129, 1920.

11. The destructive action of cow's milk diastase on various kinds of starch. F. Welzmüller. Biochem. Z., **125**, 179–186, 1921.

Experiment 18

1. Carotin, the principal natural yellow pigment of milk fat: its relations to plant carotin and the carotin of the body fat, corpus luteum, and blood serum. L. S. Palmer and C. H. Eckles. J. Biol. Chem., **17**, 191–247, 1914.

2. The detection of natural and artificial pigments in oleomargarine and butter. L. S. Palmer and W. E. Thrun. J. Ind. Eng. Chem., **8**, 614–615, 1916.

3. Lactochrome, the yellow pigment of milk whey: its probable identity with urochrome, the specific yellow pigment of normal urine. L. S. Palmer and L. H. Cooledge. J. Biol. Chem., **17**, 251–263, 1914.

4. Some little-studied constituents of milk. II. B. Bleyer and O. Kallmann. Biochem. Z., **155**, 54–79, 1925.

CHAPTER III

Experiments 19–21

1. Condition of casein and salts in milk. L. L. Van Slyke and A. W. Bosworth. J. Biol. Chem., **20**, 135–154, 1915.

2. The physico-chemical state of the proteins in cow's milk. L. S. Palmer and R. G. Scott. J. Biol. Chem., **37**, 271–284, 1919.

3. On the diffusible phosphorus of cow's milk. H. S. H. Wardlaw. J. and Proc. Roy. Soc. N. S. Wales, **48**, 253–266, 1914.

4. Colloid problems in dairy chemistry. W. Clayton. Second Report on Colloid Chemistry and its General and Industrial Applications of the British Ass'n Adv. Sci., pp. 96–112, 1919.

5. The chemistry of milk and dairy products viewed from a colloidal standpoint. L. S. Palmer. Proc. World's Dairy Con-

gress. II, 1157–1168, 1923; J. Ind. Eng. Chem., **6**, 631–635, 1924.

6. The distribution of milk salts and their chemical and physical relation to the other constituents of milk. Chas. Porcher and A. Chevallier. Le lait, **3**, 97–112, 188–200, 1923.

7. Behavior of Ca, K, Cl, and P in milk and the technic of ultrafiltration. C. Wha. Biochem. Z., **144**, 278–284, 1924.

8. An attempt to filter the enzymes of milk. Jean Piccard and Mary Rising. J. Am. Chem. Soc., **40**, 1275–1281, 1918.

CHAPTER IV

Experiment 22

1. The physical examination of butterfat. Richmond. Dairy Chemistry. 3rd Edition, pp. 269–272. Chas. Griffin and Co. Ltd., London, 1920; Theory of churning, ibid., pp. 427–432.

2. Churning. Hunziker. The Butter Industry, pp. 266–290. O. F. Hunziker, La Grange, Ill., 1920.

3. The chemistry of milk and dairy products viewed from a colloidal standpoint. L. S. Palmer. Proc. World's Dairy Congress. II, 1157–1168, 1923; J. Ind. Eng. Chem., **6**, 631–635, 1924.

4. The formation of butter. O. Rahn. Proc. World's Dairy Congress. II, 1008–1012, 1923.

5. The influence of the physical state of the fat upon the process of butter formation. W. van Dam. Milchwirtschaft. Zentr., **52**, 1–4, 13–16, 1923.

6. Phase reversal in emulsions and protoplasm. W. Seifriz. Am. J. Physiol., **66**, 124–139, 1923.

7. Emulsions. W. Seifriz. J. Phys. Chem., **29**, 587–596; 595–600; 738–749; 834–841, 1925.

8. The nature of the substances adsorbed on the surface of the fat globules in cow's milk. (Preliminary Report.) L. S. Palmer and E. Samuelsson. Proc. Soc. Exp. Biol. Med., **21**, 537–39, 1924.

9. Membrane of the fat globule of milk. K. Hattori, J. Pharm. Soc. Japan, No. 516, 123–70, 1925.

Experiment 23

1. Concerning rancidity of butter. E. S. Guthrie. J. Dairy Sci., **1**, 218–234, 1917.

2. Enzymes of milk and butter. R. W. Thatcher and A. C. Dahlberg. J. Agr. Res., **11**, 437–450, 1917.

3. The influence of various antiseptics on the activity of lipase. L. S. Palmer. J. Am. Chem. Soc., **44**, 1527–1538, 1922.

4. Tallowy butter, its causes and prevention. O. F. Hunziker and D. F. Hosman. J. Dairy Sci., **1**, 320–346, 1917.

5. Relation of the enzymes of butter to the production of tallowiness by copper salts and over-neutralization. L. S. Palmer and W. B. Combs. J. Dairy Sci., **2**, 444–453, 1919.

6. Chemical tests for the detection of rancidity. R. H. Kerr. J. Ind. Eng. Chem., **10**, 471–475, 1918.

7. The analytical detection of rancidity. R. H. Kerr and D. G. Sorber. J. Ind. Eng. Chem., **15**, 383–386, 1923.

8. Tallowiness in butterfat. G. E. Holm and G. R. Greenbank. Proc. Soc. Exp. Med., **20**, 176–177, 1922.

9. Quantitative aspect of the Kreis test. I, G. E. Holm and G. R. Greenbank. J. Ind. Eng. Chem., **15**, 1051–1053, 1924; II, ibid., **16**, 518, 1924.

10. Some factors concerned in the auto-oxidation of fats, with special reference to butterfat. G. R. Greenbank and G. E. Holm. J. Ind. Eng. Chem., **16**, 598–601, 1924.

11. The fishy flavor in butter. H. H. Sommer and B. J. Smit. Wis. Agr. Exp. Sta. Res. Bul. 57, 1–51, 1923.

Experiment 24

1. A rapid and accurate method for butter analysis, suitable for factory control work. E. F. Kohman. J. Ind. Eng. Chem., **11**, 26, 1919.

2. Comparison of the decantation method with other methods for the determination of fat in butter. O. R. Overman and S. Okimato. J. Dairy Sci., **3**, 425–429, 1920.

CHAPTER V

Experiments 25–30

1. Changes in milk brought about by the action of the digestive ferments — the rennin coagulation of milk. J. H. Kastle and N. Roberts. U. S. Hygienic Lab. Bul. 56, 348–359, 1909.

2. The Science and Practice of Cheese-making. L. L. Van Slyke and C. A. Publow, pp. 299–312, Orange Judd Co., N. Y., 1914.

3. Biochemical Catalysts in Life and Industry. J. Effront; translated by S. C. Prescott, pp. 88–113. John Wiley & Sons, N. Y., 1917.

4. Chymase. E. Zunz, in Abderhalden's "Biochemisches Handlexikon," V, pp. 618–625. J. Springer, Berlin, 1911.

5. The colloid chemistry of rennet coagulation. L. S. Palmer and G. A. Richardson. Monograph of 3rd Nat'l Colloid Symposium, pp. 112–134. Chemical Catalog Co., N. Y., 1925.

6. The conditions for the activity of the rennin enzyme. L. Michaelis and A. Mendelssohn, Biochem. Z., **58**, 315–328, 1914.

7. The action of hydrogen ions on the clotting of milk. Rôle of acidity on the retractility of the clot. N. L. Cosmovici. Bull. soc. chim. biol., **7**, 124–152, 153–154, 1925.

8. Rennet action. W. Grimmer and M. Krüger. Milchwirtschaft. Forsch., **2**, 457–481, 1925.

CHAPTER VI

Experiments 31–33

1. Chemical changes in cheese-ripening. Van Slyke and Publow. The Science and Practice of Cheese-making, pp. 327–378. Orange Judd Co., N. Y., 1914.

2. Following the casein decomposition of cheese-ripening by means of an ultrafilter. C. Mannich and S. Wipperling. Z. Nahr. Genussm., **40**, 20–23, 1920.

3. The nitrogen compounds of skimmed milk cheese. E. Winterstein and O. Huppert. Biochem. Z., **141**, 193–221, 1923.

4. The chemistry of cheese-ripening. I. The degradation of

protein during ripening of Tilsit cheese. W. Grimmer. Forsch. Geb. Milkwirtsch. Molkereiwes., **2**, 119–23, 1922.

5. Disappearance of sugar in cheese and source of lactic acid. S. K. Suzuki, E. G. Hastings, and E. B. Hart. J. Biol. Chem., **7**, 439, 442, 1909–10.

6. Composition of brine-soluble compounds of cheese. L. L. Van Slyke and A. W. Bosworth. N. Y. Agr. Exp. Sta. Tech. Bul. 26, 28–32, 1912.

CHAPTER VII

Experiment 34

1. History of dried milk, methods of preparation, and conditions of manufacture. Physical and chemical characters of dried milk. F. J. H. Coutts. Report to the Local Government Board N. S. No. 116. (Food Report 24) pp. 3–13, 23–42, 1920.

2. On the examination of milk powders at the Government laboratory. J. J. Dobbie. Report to the Local Government Board N. S. No. 116. (Food Report 24) pp. 157–184, 1920.

3. A study of the chemical and physical properties of remade milk. L. S. Palmer and C. D. Dahle. J. Dairy Sci., **5**, 1–13, 1922.

4. Structure of powdered milk and its possible relation to the keeping quality of whole-milk powders. L. S. Palmer and C. D. Dahle. J. Dairy Sci., **5**, 240–245, 1922.

5. A color test for " remade " milk and cream. O. L. Evenson. J. Dairy Sci., **5**, 97–101, 1922.

6. Results of further investigations of remade milk and milk powder. O. L. Evenson. Internat'l Assoc. Dairy and Milk Insp. Ann. Rep., **11**, 241–246, 1922.

7. Remade milk. O. L. Evenson. Internat'l Assoc. Dairy and Milk Insp. Ann. Rep., **12**, 354–371, 1923.

8. The keeping quality of butterfat, with special reference to milk powder. G. E. Holm and G. R. Greenbank. Proc. World's Dairy Congress. II, 1253–1265, 1923.

9. The keeping quality of dry milk. G. C. Supplee. Proc. World's Dairy Congress. II, 1248–1253, 1923.

10. Some factors affecting the keeping quality of whole-milk

powders. C. D. Dahle and L. S. Palmer. J. Dairy Sci., **7**, 40–57, 1924.

11. The effect of storage on the peroxidase activity of whole-milk powders. C. D. Dahle and L. S. Palmer. J. Dairy Sci., **7**, 141–146, 1924.

CHAPTER VIII

Experiment 35

1. The analysis of dairy products. Official and tentative methods of analysis of the A. O. A. C., pp. 259–280, 1925.

2. The technical control of dairy products. Timothy Mojonnier and H. C. Troy. Mojonnier Bros., Chicago, 1922.

3. The preservation of milk for chemical analysis. L. S. Palmer. Mo. Agr. Exp. Sta. Res. Bul. 34, 3–31, 1919.

Experiment 36

1. A volumetric method for the determination of casein in milk. L. L. Van Slyke and A. W. Bosworth. N. Y. Agr. Exp. Sta. Tech. Bul. 10, 231–249, 1909.

Experiment 37

1. The freezing point of milk considered in its relation to the detection of added water. G. W. Monier-Williams. Report to Local Gov. Board N. S. No. 103 (Food Report 22), pp. 1–32, 1914.

2. Application of the cryoscopic method for determining added water in milk. J. T. Keister. J. Ind. Eng. Chem., **9**, 862–865, 1917.

3. The cryoscopy of milk. J. Hortvet. J. Ind. Eng. Chem., **13**, 198–208, 1921.

4. Cryoscopy of milk. E. M. Bailey. Conn. State Sta. Bul. 236, 251–271, 1922.

5. Cryoscopy of milk. E. M. Bailey. Conn. State Sta. Bul. 248, 411–415, 1923.

APPENDIX

TABLE I

INTERNATIONAL TABLE OF ATOMIC WEIGHTS OF THE CHEMICAL ELEMENTS
1925

Name	Symbol	Principal Valence	Atomic Weight
Aluminum	Al	3	26.97
Antimony	Sb	3 or 5	121.77
Argon	A	0	39.91
Arsenic	As	3 or 5	74.96
Barium	Ba	2	137.37
Beryllium	Be	2	9.02
Bismuth	Bi	3 or 5	209.00
Boron	B	3	10.82
Bromine	Br	1	79.916
Cadmium	Cd	2	112.41
Calcium	Ca	2	40.07
Carbon	C	2 or 4	12.000
Cerium	Ce	4 or 3	140.25
Cesium	Cs	1	132.81
Chlorine	Cl	1	35.457
Chromium	Cr	2, 3, or 6	52.01
Cobalt	Co	2 or 3	58.94
Columbium	Cb	3 or 5	93.1
Copper	Cu	1 or 2	63.57
Dysprosium	Dy	3	162.52
Erbium	Er	3	167.7
Europium	Eu	3	152.0
Fuorline	F	1	19.00
Gadolinium	Gd	3	157.26
Gallium	Ga	3	69.72
Germanium	Ge	4	72.60
Gold	Au	1 or 3	197.2
Helium	He	0	4.00
Holmium	Ho	3	163.4
Hydrogen	H	1	1.008
Indium	In	3	114.8
Iodine	I	1	126.932
Iridium	Ir	3 or 4	193.1
Iron	Fe	2 or 3	55.84
Krypton	Kr	0	82.9
Lanthanum	La	3	138.90
Lead	Pb	2 or 4	207.20
Lithium	Li	1	6.940
Lutecium	Lu	3	175.0
Magnesium	Mg	2	24.32
Manganese	Mn	2, 4, 6, or 7	54.93
Mercury	Hg	1 or 2	200.61

TABLE I. — *Continued*

Name	Symbol	Principal Valence	Atomic Weight
Molybdenum	Mo	3, 4, or 6	96.0
Neodymium	Nd	3	144.27
Neon	Ne	0	20.2
Nickel	Ni	2 or 3	58.69
Nitrogen	N	3 or 5	14.008
Osmium	Os	2, 3, 4, or 8	190.8
Oxygen	O	2	16.000
Palladium	Pd	2 or 4	106.7
Phosphorus	P	3 or 5	31.027
Platinum	Pt	2 or 4	195.23
Potassium	K	1	39.096
Praseodymium	Pr	3	140.92
Radium	Ra	2	225.95
Radon	Rn	.....	222
Rhodium	Rh	3	102.91
Rubidium	Rb	1	85.44
Ruthenium	Ru	3, 4, 6, or 8	101.7
Samarium	Sm	3	150.43
Scandium	Sc	3	45.10
Selenium	Se	2, 4, or 6	79.2
Silicon	Si	4	28.06
Silver	Ag	1	107.880
Sodium	Na	1	22.997
Strontium	Sr	2	87.63
Sulfur	S	2, 4, or 6	32.064
Tantalum	Ta	5	181.5
Tellurium	Te	2, 4, or 6	127.5
Terbium	Tb	3	159.2
Thallium	Tl	1 or 3	204.39
Thorium	Th	4	232.15
Thulium	Tm	3	169.4
Tin	Sn	2 or 4	118.70
Titanium	Ti	3 or 4	48.1
Tungsten	W	6	184.0
Uranium	U	4 or 6	238.17
Vanadium	V	3 or 5	50.96
Xenon	Xe	0	130.2
Ytterbium	Yb	3	173.6
Yttrium	Y	3	88.9
Zinc	Zn	2	65.38
Zirconium	Zr	4	91

TABLE II

CENTIGRADE — FAHRENHEIT CONVERSION TABLE

°F. = (°C. × 1.8) + 32. °C. = (°F. − 32) ÷ 1.8

0° C.	0	+1	+2	+3	+4	+5	+6	+7	+8	+9
−20	−4.0	−2.2	−0.4	1.4	3.8	5.0	6.8	8.6	10.4	12.2
−10	14.0	15.8	17.6	19.4	21.2	23.0	24.8	26.6	28.4	30.2
0	32.0	33.8	35.6	37.4	39.2	41.0	42.8	44.6	46.4	48.2
10	50.0	51.8	53.6	55.4	57.2	59.0	60.8	62.6	64.4	66.2
20	68.0	69.8	71.6	73.4	75.2	77.0	78.8	80.6	82.4	84.2
30	86.0	87.8	89.6	91.4	93.2	95.0	96.8	98.6	100.4	102.2
40	104.0	105.8	107.6	109.4	111.2	113.0	114.8	116.6	118.4	120.2
50	122.0	123.8	125.6	127.4	129.2	121.0	132.8	134.6	136.4	138.2
60	140.0	141.8	143.6	145.4	147.2	149.0	150.8	152.6	154.4	156.2
70	158.0	159.8	161.6	163.4	165.2	167.0	168.8	170.6	172.4	174.2
80	176.0	177.8	179.6	181.4	183.2	185.0	186.8	188.6	190.4	192.2
90	194.0	195.8	197.6	199.4	201.2	203.0	204.8	206.6	208.4	210.2
100	212.0									

In this table the Centigrade temperatures are given at the left and top and the Fahrenheit temperatures in the body of the table. To use the −0 Centrigrade find the algebraic sum of the left and top. For example, −15° C. in terms of °F. is found opposite the −20 line and under the +5 column.

TABLE III

TABLE FOR CORRECTING SPECIFIC GRAVITY OF WHOLE MILK TO 60° F. (AFTER RACE)

Temp. Deg. Fahr.	DEGREES OF LACTOMETER																Temp. Deg. Fahr.
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
45	19.0	19.9	20.9	21.9	22.9	23.8	24.8	25.8	26.7	27.7	28.6	29.5	30.4	31.4	32.3	33.2	45
46	19.0	20.0	21.0	22.0	22.9	23.9	24.9	25.9	26.8	27.8	28.7	29.6	30.5	31.4	32.4	33.3	46
47	19.1	20.0	21.0	22.0	23.0	24.0	24.9	25.9	26.8	27.8	28.7	29.6	30.5	31.5	32.5	33.4	47
48	19.1	20.1	21.1	22.1	23.1	24.0	25.0	26.0	26.9	27.9	28.8	29.7	30.6	31.6	32.6	33.5	48
49	19.2	20.2	21.2	22.2	23.2	24.1	25.1	26.1	27.0	28.0	28.9	29.8	30.7	31.7	32.7	33.6	49
50	19.2	20.2	21.2	22.2	23.2	24.1	25.1	26.1	27.0	28.0	29.0	29.9	30.9	31.8	32.8	33.7	50
51	19.3	20.3	21.3	22.3	23.3	24.2	25.2	26.2	27.1	28.1	29.1	30.0	31.0	31.9	32.9	33.8	51
52	19.4	20.3	21.3	22.3	23.3	24.3	25.2	26.2	27.2	28.2	29.1	30.1	31.1	32.0	33.0	33.9	52
53	19.4	20.4	21.4	22.4	23.4	24.4	25.3	26.3	27.3	28.3	29.2	30.2	31.2	32.1	33.1	34.0	53
54	19.5	20.5	21.5	22.5	23.5	24.5	25.4	26.4	27.4	28.4	29.3	30.3	31.3	32.3	33.3	34.2	54
55	19.6	20.6	21.6	22.6	23.6	24.6	25.5	26.5	27.5	28.5	29.4	30.4	31.4	32.4	33.4	34.3	55
56	19.7	20.7	21.7	22.7	23.6	24.6	25.6	26.6	27.6	28.6	29.6	30.5	31.5	32.5	33.5	34.4	56
57	19.8	20.8	21.8	22.8	23.7	24.7	25.7	26.7	27.7	28.7	29.7	30.6	31.6	32.6	33.6	34.5	57
58	19.9	20.9	21.9	22.8	23.8	24.8	25.8	26.8	27.8	28.8	29.8	30.8	31.7	32.7	33.7	34.7	58
59	19.9	20.9	21.9	22.9	23.9	24.9	25.9	26.9	27.9	28.9	29.9	30.9	31.9	32.9	33.9	34.8	59
60	20.0	21.0	22.0	23.0	24.0	25.0	26.0	27.0	28.0	29.0	30.0	31.0	32.0	33.0	34.0	35.0	60
61	20.1	21.1	22.1	23.1	24.1	25.1	26.1	27.1	28.1	29.1	30.1	31.1	32.1	33.1	34.1	35.1	61
62	20.2	21.2	22.2	23.2	24.2	25.2	26.2	27.3	28.3	29.3	30.3	31.3	32.3	33.3	34.3	35.3	62
63	20.2	21.3	22.3	23.3	24.3	25.3	26.3	27.4	28.4	29.4	30.4	31.4	32.5	33.5	34.5	35.5	63
64	20.3	21.4	22.4	23.4	24.4	25.4	26.5	27.5	28.5	29.5	30.5	31.5	32.6	33.6	34.6	35.6	64
65	20.4	21.5	22.5	23.5	24.5	25.5	26.6	27.6	28.6	29.6	30.7	31.7	32.7	33.8	34.8	35.8	65
66	20.5	21.6	22.6	23.6	24.6	25.6	26.7	27.7	28.7	29.8	30.8	31.8	32.9	33.9	34.9	35.9	66
67	20.6	21.7	22.7	23.7	24.7	25.7	26.8	27.8	28.8	29.9	30.9	32.0	33.0	34.0	35.0	36.1	67
68	20.7	21.8	22.8	23.8	24.9	25.9	27.0	28.0	29.0	30.1	31.1	32.1	33.2	34.2	35.2	36.2	68
69	20.9	22.0	23.0	24.0	25.0	26.0	27.1	28.1	29.1	30.2	31.2	32.2	33.3	34.3	35.3	36.3	69
70	21.0	22.1	23.1	24.1	25.1	26.1	27.2	28.2	29.2	30.3	31.3	32.4	33.4	34.5	35.5	36.5	70
71	21.1	22.2	23.2	24.2	25.2	26.2	27.3	28.3	29.4	30.4	31.5	32.5	33.6	34.6	35.6	36.7	71
72	21.2	22.3	23.3	24.3	25.3	26.4	27.4	28.4	29.5	30.5	31.6	32.6	33.7	34.7	35.8	36.8	72
73	21.3	22.4	23.4	24.4	25.5	26.5	27.5	28.6	29.7	30.7	31.8	32.8	33.9	34.9	36.0	37.0	73
74	21.5	22.5	23.5	24.6	25.6	26.6	27.7	28.7	29.8	30.9	31.9	33.0	34.1	35.1	36.2	37.2	74
75	21.6	22.6	23.7	24.7	25.7	26.8	27.8	28.9	29.9	31.0	32 1	33.2	34.3	35.3	36.4	37.4	75

Do not use this Table for skim milk. Instead subtract 0.1 lactometer degrees from the observed reading for each degree F. below 60°F., and add 0.14 lactometer degrees for each degree F. above 60° F. Examples: (1) If the observed temperature is 72° and the observed lactometer reading is 33.9, the corrected lactometer reading for 60° F. will be $33.9 + (12 \times 0.14) = 35.58$. (2) If the observed temperature is 52° F. and the observed lactometer reading is 36.5, the corrected lactometer reading for 60° F. will be $36.4 - (8 \times 0.1) = 35.6$.

TABLE IV

Table for Determining Total Solids in Milk from any Given Specific Gravity and Percentage of Fat. (Shaw and Eckles)

Per-centage of fat	Lactometer reading at 60° F. (Quevenne degrees)										
	26	27	28	29	30	31	32	33	34	35	36
	Per cent total solids	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*
2.00	8.90	9.15	9.40	9.65	9.90	10.15	10.40	10.66	10.91	11.16	11.41
2.05	8.96	9.21	9.46	9.71	9.96	10.21	10.46	10.72	10.97	11.22	11.47
2.10	9.02	9.27	9.52	9.77	10.02	10.27	10.52	10.78	11.03	11.28	11.53
2.15	9.08	9.33	9.58	9.83	10.08	10.33	10.58	10.84	11.09	11.34	11.59
2.20	9.14	9.39	9.64	9.89	10.14	10.39	10.64	10.90	11.15	11.40	11.65
2.25	9.20	9.45	9.70	9.95	10.20	10.45	10.70	10.96	11.21	11.46	11.71
2.30	9.26	9.51	9.76	10.01	10.26	10.51	10.76	11.02	11.27	11.52	11.77
2.35	9.32	9.57	9.82	10.07	10.32	10.57	10.82	11.08	11.33	11.58	11.83
2.40	9.38	9.63	9.88	10.13	10.38	10.63	10.88	11.14	11.39	11.64	11.89
2.45	9.44	9.69	9.94	10.19	10.44	10.69	10.94	11.20	11.45	11.70	11.95
2.50	9.50	9.75	10.00	10.25	10.50	10.75	11.00	11.26	11.51	11.76	12.01
2.55	9.56	9.81	10.06	10.31	10.56	10.81	11.06	11.32	11.57	11.82	12.07
2.60	9.62	9.87	10.12	10.37	10.62	10.87	11.12	11.38	11.63	11.88	12.13
2.65	9.68	9.93	10.18	10.43	10.68	10.93	11.18	11.44	11.69	11.94	12.19
2.70	9.74	9.99	10.24	10.49	10.74	10.99	11.24	11.50	11.75	12.00	12.25
2.75	9.80	10.05	10.30	10.55	10.80	11.05	11.31	11.56	11.81	12.06	12.31
2.80	9.86	10.11	10.36	10.61	10.86	11.11	11.37	11.62	11.87	12.12	12.37
2.85	9.92	10.17	10.42	10.67	10.92	11.17	11.43	11.68	11.93	12.18	12.43
2.90	9.98	10.23	10.48	10.73	10.98	11.23	11.49	11.74	11.99	12.24	12.49
2.95	10.04	10.29	10.54	10.79	11.04	11.30	11.55	11.80	12.05	12.30	12.55
3.00	10.10	10.35	10.60	10.85	11.10	11.36	11.61	11.86	12.11	12.36	12.61
3.05	10.16	10.41	10.66	10.91	11.17	11.42	11.67	11.92	12.17	12.42	12.68
3.10	10.22	10.47	10.72	10.97	11.23	11.48	11.73	11.98	12.23	12.48	12.74
3.15	10.28	10.53	10.78	11.03	11.29	11.54	11.79	12.04	12.29	12.55	12.80
3.20	10.34	10.59	10.84	11.09	11.35	11.60	11.85	12.10	12.35	12.61	12.86
3.25	10.40	10.65	10.90	11.16	11.41	11.66	11.91	12.16	12.42	12.67	12.92
3.30	10.46	10.71	10.96	11.22	11.47	11.72	11.97	12.22	12.48	12.73	12.98
3.35	10.52	10.77	11.03	11.28	11.53	11.78	12.03	12.28	12.54	12.79	13.04
3.40	10.58	10.83	11.09	11.34	11.59	11.84	12.09	12.34	12.60	12.85	13.10
3.45	10.64	10.89	11.15	11.40	11.65	11.90	12.15	12.40	12.66	12.91	13.16
3.50	10.70	10.95	11.21	11.46	11.71	11.96	12.21	12.46	12.72	12.97	13.22
3.55	10.76	11.02	11.27	11.52	11.77	12.02	12.27	12.52	12.78	13.03	13.28
3.60	10.82	11.08	11.33	11.58	11.83	12.08	12.33	12.58	12.84	13.09	13.34
3.65	10.88	11.14	11.39	11.64	11.89	12.14	12.39	12.64	12.90	13.15	13.40
3.70	10.94	11.20	11.45	11.70	11.95	12.20	12.45	12.70	12.96	13.21	13.46
3.75	11.00	11.26	11.51	11.76	12.01	12.26	12.51	12.76	13.02	13.27	13.52
3.80	11.06	11.32	11.57	11.82	12.07	12.32	12.57	12.82	13.08	13.33	13.58
3.85	11.12	11.38	11.63	11.88	12.13	12.38	12.63	12.88	13.14	13.39	13.64
3.90	11.18	11.44	11.69	11.94	12.19	12.44	12.69	12.94	13.20	13.45	13.70
3.95	11.24	11.50	11.75	12.00	12.25	12.50	12.75	13.00	13.26	13.51	13.77
4.00	11.30	11.56	11.81	12.06	12.31	12.56	12.81	13.06	13.32	13.57	13.83
4.05	11.36	11.62	11.87	12.12	12.37	12.62	12.87	13.12	13.38	13.63	13.89
4.10	11.42	11.68	11.93	12.18	12.43	12.68	12.93	13.18	13.44	13.69	13.95
4.15	11.48	11.74	11.99	12.24	12.49	12.74	12.99	13.25	13.50	13.76	14.01
4.20	11.54	11.80	12.05	12.30	12.55	12.80	13.05	13.31	13.56	13.82	14.07
4.25	11.60	11.86	12.11	12.36	12.61	12.86	13.12	13.37	13.62	13.88	14.13
4.30	11.66	11.92	12.17	12.42	12.67	12.92	13.18	13.43	13.68	13.94	14.19
4.35	11.72	11.98	12.23	12.48	12.73	12.98	13.24	13.49	13.74	14.00	14.25
4.40	11.78	12.04	12.29	12.54	12.79	13.04	13.30	13.55	13.80	14.06	14.31
4.45	11.84	12.10	12.35	12.60	12.85	13.10	13.36	13.61	13.86	14.12	14.37
4.50	11.90	12.16	12.41	12.66	12.91	13.16	13.42	13.67	13.92	14.18	14.43
4.55	11.97	12.22	12.47	12.72	12.97	13.22	13.48	13.73	13.98	14.24	14.49
4.60	12.03	12.28	12.53	12.78	13.03	13.28	13.54	13.79	14.04	14.30	14.55
4.65	12.09	12.34	12.59	12.84	13.09	13.34	13.60	13.85	14.10	14.36	14.61
4.70	12.15	12.40	12.65	12.90	13.15	13.40	13.66	13.91	14.16	14.42	14.67
4.75	12.21	12.46	12.71	12.96	13.21	13.46	13.72	13.97	14.22	14.48	14.73
4.80	12.27	12.52	12.77	13.02	13.27	13.52	13.78	14.03	14.28	14.54	14.79
4.85	12.33	12.58	12.83	13.08	13.33	13.58	13.84	14.09	14.34	14.60	14.85
4.90	12.39	12.64	12.89	13.14	13.39	13.64	13.90	14.15	14.40	14.66	14.91
4.95	12.45	12.70	12.95	13.20	13.45	13.70	13.96	14.21	14.46	14.72	14.97

TABLE IV — *Continued*

TABLE FOR DETERMINING TOTAL SOLIDS IN MILK FROM ANY GIVEN SPECIFIC GRAVITY AND PERCENTAGE OF FAT. (SHAW AND ECKLES)

Per-centage of fat	Lactometer reading at 60° F. (Quevenne degrees).										
	26	27	28	29	30	31	32	33	34	35	36
	Per cent total solids	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*	*Per cent total solids*
5.00	12.51	12.76	13.01	13.26	13.51	13.76	14.02	14.27	14.52	14.78	15.03
5.05	12.57	12.82	13.07	13.32	13.57	13.83	14.08	14.33	14.58	14.84	15.09
5.10	12.63	12.88	13.13	13.38	13.63	13.89	14.14	14.39	14.64	14.90	15.15
5.15	12.69	12.94	13.19	13.44	13.69	13.95	14.20	14.45	14.70	14.96	15.21
5.20	12.75	13.00	13.25	13.50	13.75	14.01	14.26	14.51	14.76	15.02	15.27
5.25	12.81	13.06	13.31	13.56	13.81	14.07	14.32	14.57	14.82	15.08	15.33
5.30	12.87	13.12	13.37	13.62	13.87	14.13	14.38	14.63	14.88	15.14	15.39
5.35	12.93	13.18	13.43	13.68	13.93	14.19	14.44	14.70	14.95	15.20	15.45
5.40	12.99	13.24	13.49	13.74	14.00	14.25	14.50	14.76	15.01	15.26	15.51
5.45	13.05	13.30	13.55	13.80	14.06	14.31	14.56	14.82	15.07	15.32	15.57
5.50	13.11	13.36	13.61	13.86	14.12	14.37	14.62	14.88	15.13	15.38	15.63
5.55	13.17	13.42	13.67	13.93	14.18	14.43	14.69	14.94	15.19	15.44	15.69
5.60	13.23	13.48	13.73	13.99	14.24	14.49	14.75	15.00	15.25	15.50	15.75
5.65	13.29	13.54	13.79	14.05	14.30	14.55	14.81	15.06	15.31	15.56	15.81
5.70	13.35	13.60	13.85	14.11	14.36	14.61	14.87	15.12	15.37	15.62	15.87
5.75	13.41	13.66	13.91	14.17	14.42	14.68	14.93	15.18	15.43	15.68	15.93
5.80	13.47	13.72	13.97	14.23	14.48	14.74	14.99	15.24	15.49	15.74	15.99
5.85	13.53	13.78	14.04	14.29	14.54	14.80	15.05	15.30	15.55	15.80	16.06
5.90	13.59	13.84	14.10	14.35	14.60	14.86	15.11	15.36	15.61	15.86	16.12
5.95	13.65	13.90	14.16	14.41	14.66	14.92	15.17	15.42	15.67	15.92	16.18
6.00	13.71	13.96	14.22	14.47	14.72	14.98	15.23	15.48	15.73	15.98	16.24
6.05	13.77	14.02	14.28	14.53	14.78	15.04	15.29	15.54	15.79	16.04	16.30
6.10	13.83	14.08	14.34	14.59	14.84	15.10	15.35	15.60	15.85	16.10	16.35
6.15	13.89	14.14	14.40	14.65	14.90	15.16	15.41	15.66	15.91	16.16	16.42
6.20	13.95	14.20	14.46	14.71	14.96	15.22	15.47	15.72	15.97	16.22	16.48
6.25	14.01	14.26	14.52	14.77	15.02	15.28	15.53	15.78	16.03	16.28	16.54
6.30	14.07	14.32	14.58	14.83	15.08	15.34	15.59	15.84	16.09	16.34	16.60
6.35	14.13	14.38	14.64	14.90	15.14	15.40	15.65	15.90	16.15	16.40	16.66
6.40	14.19	14.44	14.70	14.96	15.20	15.46	15.71	15.96	16.21	16.46	16.72
6.45	14.25	14.50	14.76	15.02	15.26	15.52	15.77	16.02	16.27	16.52	16.78
6.50	14.31	14.56	14.82	15.08	15.32	15.58	15.83	16.08	16.33	16.58	16.84
6.55	14.37	14.62	14.88	15.14	15.38	15.64	15.89	16.14	16.39	16.64	16.90
6.60	14.43	14.68	14.94	15.20	15.44	15.70	15.95	16.20	16.45	16.70	16.96
6.65	14.49	14.74	15.00	15 26	15.50	15.76	16.01	16.26	16.51	16.76	17.02
6.70	14.55	14.80	15.06	15.32	15.56	15.82	16.07	16.32	16.57	16.82	17.08
6.75	14.61	14.86	15.12	15.38	15.62	15.88	16.13	16.38	16.63	16.88	17.14
6.80	14.67	14.92	15.18	15.44	15.68	15.94	16.19	16.44	16.69	16.94	17.20
6.85	14.73	14.98	15.24	15.50	15.74	16.00	16.25	16.50	16.75	17.00	17.26
6.90	14.79	15.04	15.30	15.56	15.80	16.06	16.31	16.56	16.81	17.06	17.32
6.95	14.85	15.10	15.36	15.62	15.86	16.12	16.37	16.62	16.87	17.12	17.38

PROPORTIONAL PARTS

Lactometer fraction	Fraction to be added to total solids	Lactometer fraction	Fraction to be added to total solids	Lactometer fraction	Fraction to be added to total solids
0.1	0.03	0.4	0.10	0.7	0.18
.2	.05	.5	.13	.8	.20
.3	.08	.6	.15	.9	.23

This table is used as follows:

" If the specific gravity as expressed in Quevenne degrees is a whole number, the percentage of total solids is found at the intersection of the vertical column headed by this number with the horizontal column corresponding to the percentage of fat.

" If the specific gravity as expressed in Quevenne degrees is a whole number and a decimal, the percentage of total solids corresponding to the whole number is first found and to this is added the fraction found opposite the tenth under ' Proportional Parts.' Two examples may suffice for illustration: (1) Fat, 3.8 per cent; specific gravity, 32. Under the column headed 32 we find 12.57 per cent, corresponding to 3.8 per cent fat. (2) Fat, 3.8 per cent; specific gravity, 32.5. The percentage of total solids corresponding to this percentage of fat and a specific gravity of 32 is 12.57. Under ' Proportional Parts ' the fraction 0.13 appears opposite 0.5. This added to 12.57 makes 12.70, which is the desired percentage."

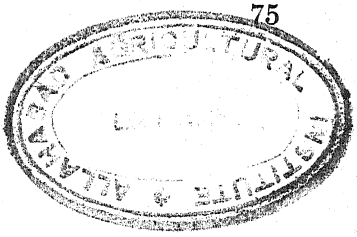


TABLE V

GENERAL RELATION BETWEEN *pH*, C_{H^+}, C_{OH^-}, NORMALITY OF HCl AND NaOH SOLUTIONS

pH	C_{H^+}	C_{OH^-}	Conc. HCl	Conc. NaOH
1.0	1.0×10^{-1}	1.0×10^{-13}	0.10 *N*	.....
1.4	.4	2.5	.04	.....
1.7	.2	5.0	.02	.....
2.0	1.0×10^{-2}	1.0×10^{-12}	.01	.....
2.1	.8	1.3	.008	.....
2.4	.4	2.5	.004	.....
2.7	.2	5.0	.002	.....
3.0	1.0×10^{-3}	1.0×10^{-11}	.001	.....
4.0	1.0×10^{-4}	1.0×10^{-10}	.0001	.....
5.0	1.0×10^{-5}	1.0×10^{-9}	.00001	.....
6.0	1.0×10^{-6}	1.0×10^{-8}	.000001	.....
8.0	1.0×10^{-8}	1.0×10^{-6}	.....	.000001 *N*
9.0	1.0×10^{-9}	1.0×10^{-5}	.....	.00001
10.0	1.0×10^{-10}	1.0×10^{-4}	.....	.0001
11.0	1.0×10^{-11}	1.0×10^{-3}	.....	.001
11.1	.8	1.3	.....	.00125
11.4	.4	2.5	.....	.0025
12.0	1.0×10^{-12}	1.0×10^{-2}	.....	.01
12.4	.4	2.5	.....	.025
12.7	.2	5.0	.....	.05
13.0	1.0×10^{-13}	1.0×10^{-1}	.....	.10

TABLE VI

pH RANGE, COLOR AND COMPOSITION OF VARIOUS INDICATOR SOLUTIONS

Indicator	*pH* Range	Color		Composition of 100 cc. of Indicator
		Acid	Alkaline	
Thymol blue.....	1.2–2.8	Red	Yellow	.04 g. + 1.7 cc. *N*/20 NaOH
Brom phenol blue....	3.0–4.6	Yellow	Blue	.04 g. + 1.2 cc. *N*/10 NaOH
Methyl orange.....	3.1–4.4	Red	Yellow	.10 g. in water
Congo red.....	3.0–5.0	Blue	Red	.5 g. in aqueous solution containing 10 cc. 95 per cent alcohol
Methyl red.....	4.4–6.0	Red	Yellow	.05 g. in 50 per cent alcohol
Cochineal.....	4.8–6.2	Yellow	Lilac	1.0 g. in 20 per cent alcohol
Litmus.....	4.5–8.3	Red	Blue	1.5 g. in water. Make very slightly acid or alkaline as desired
Brom cresol purple...	5.2–6.8	Yellow	Purple	.04 g. + 1.5 cc. *N*/20 NaOH
Brom thymol blue....	6.0–7.6	Yellow	Blue	.04 g. + 1.3 cc. *N*/20 NaOH
Thymol blue.....	8.0–9.6	Yellow	Blue	.04 g. + 1.7 cc. *N*/20 NaOH
Phenolphthalein.....	8.5–10.0	Colorless	Red	.5–1.0 g. in 95 per cent alcohol for titration work; .04 per cent sol. for *pH* work
Thymolphthalein.....	9.3–10.5	Colorless	Blue	Same as phenolphthalein

TABLE VII

PHOSPHATE BUFFERS (SÖRENSEN)

Na_2HPO_4	KH_2PO_4	*pH*
cc. *M*/15	cc. *M*/15	
0.25	9.75	5.288
0.5	9.5	5.589
1.0	9.0	5.906
2.0	8.0	6.239
3.0	7.0	6.468
4.0	6.0	6.643
5.0	5.0	6.813
6.0	4.0	6.979
7.0	3.0	7.168
8.0	2.0	7.381
9.0	1.0	7.731
9.5	0.5	8.043

The phosphate solutions are prepared as follows: 11.876 g. of Na_2HPO_4, 2 H_2O are dissolved in 1 liter of water. Ordinary crystals of the secondary sodium phosphate contain 12 mols of water. These crystals on exposure to the air for two weeks lose most of this water and change to the Na_2HPO_4, 2 H_2O. The salt should be sufficiently pure to give a clear aqueous solution which will give no test for chloride or sulfate. The primary phosphate solution is prepared by dissolving 9.078 g. of pure KH_2PO_4, free from chlorides and sulfates, in 1 liter of water.

TABLE VIII

TABLE FOR CALCULATING THE AMOUNT OF WATER ADDED TO MILK, BASED ON THE FREEZING POINT (AFTER WINTER)

Temperature observed. Degrees below zero	Ccs. of added water in 1 liter of sample	Temperature observed. Degrees below zero	Ccs. of added water in 1 liter of sample	Temperature observed. Degrees below zero	Ccs. of added water in 1 liter of sample	Temperature observed. Degrees below zero	Ccs. of added water in 1 liter of sample	Temperature observed. Degrees below zero	Ccs. of added water in 1 liter of sample
.550	0	.510	72.7	.470	145.4	.430	218.2	.390	290.9
.549	1.8	.509	74.5	.469	147.3	.429	220.0	.389	292.7
.548	3.6	.508	76.4	.468	149.1	.428	221.8	.388	294.5
.547	5.4	.507	78.2	.467	150.9	.427	223.6	.387	296.4
.546	7.3	.506	80.0	.466	152.7	.426	225.4	.386	298.2
.545	9.1	.505	81.8	.465	154.5	.425	227.3	.385	300.0
.544	10.9	.504	83.6	.464	156.3	.424	229.1	.384	301.8
.543	12.7	.503	85.4	.463	158.2	.423	230.9	.383	303.6
.542	14.5	.502	87.3	.462	160.0	.422	232.7	.382	305.4
.541	16.3	.501	89.1	.461	161.8	.421	234.5	.381	307.3
.540	18.2	.500	90.9	.460	163.6	.420	236.4	.380	309.1
.539	20.0	.499	92.7	.459	165.4	.419	238.2	.379	310.9
.538	21.8	.498	94.5	.458	167.3	.418	240.0	.378	312.7
.537	23.6	.497	96.4	.457	169.1	.417	241.8	.377	314.5
.536	25.4	.496	98.2	.456	170.9	.416	243.6	.376	316.4
.535	27.2	.495	100.0	.455	172.7	.415	245.4	.375	318.2
.534	29.1	.494	101.8	.454	174.5	.414	247.3	.374	320.0
.533	30.9	.493	103.6	.453	176.4	.413	249.1	.373	321.8
.532	32.7	.492	105.4	.452	178.2	.412	250.9	.372	323.6
.531	34.5	.491	107.3	.451	180.0	.411	252.7	.371	325.4
.530	36.4	.490	109.1	.450	181.8	.410	254.5	.370	327.3
.529	38.2	.489	110.9	.449	183.6	.409	256.4	.369	329.1
.528	40.0	.488	112.7	.448	185.4	.408	258.2	.368	330.9
.527	41.8	.487	114.5	.447	187.3	.407	260.0	.367	332.7
.526	43.6	.486	116.4	.446	189.1	.406	261.8	.366	334.5
.525	45.4	.485	118.2	.445	190.9	.405	263.6	.365	336.4
.524	47.3	.484	120.0	.444	192.7	.404	265.4	.364	338.2
.523	49.1	.483	121.8	.443	194.5	.403	267.3	.363	340.0
.522	50.9	.482	123.6	.442	196.4	.402	269.1	.362	341.8
.521	52.7	.481	125.4	.441	198.2	.401	270.9	.361	343.6
.520	54.5	.480	127.3	.440	200.0	.400	272.7	.360	345.4
.519	56.3	.479	129.1	.439	201.8	.399	274.5	.359	347.3
.518	58.2	.478	130.9	.438	203.6	.398	276.4	.358	349.1
.517	60.0	.477	132.7	.437	205.4	.397	278.2	.357	350.9
.516	61.8	.476	134.5	.436	207.3	.396	280.0	.356	352.7
.515	63.6	.475	136.4	.435	209.1	.395	281.8	.355	354.5
.514	65.4	.474	138.2	.434	210.9	.394	283.6	.354	356.4
.513	67.3	.473	140.0	.433	212.7	.393	285.4	.353	358.2
.512	69.1	.472	141.8	.432	214.5	.392	287.3	.352	360.0
.511	70.9	.471	143.7	.431	216.4	.391	289.1	.351	361.8

TABLE IX

AVERAGE COMPOSITION AND NORMAL RANGE OF COW'S MILK

Constituent	Average concentration	Normal range
	per cent	
Water	87.10	82.6–91.1
Total solids	12.90	8.9–17.4
Lactose	4.90	4.4–5.6
Casein	2.30	2.0–7.6*
Lactalbumin	0.60	0.3–2.0*
Globulin	0.05	0.04–17.5*
Non-protein nitrogenous substances	0.25	.....
Fat	3.90	2.0–7.0
Composition of Fat		
Glycerol	0.40	.....
Unsaponifiable	0.04	.....
Oleic acid	31.89	25.3–40.3
Myristic acid	19.78	15.6–22.6
Palmitic acid	15.16	5.8–22.9
Stearic acid	14.91	7.8–20.4
Lauric acid	5.85	4.5–7.7
Butyric acid	2.93	2.2–4.2
Caproic acid	1.90	1.3–2.4
Capric acid	1.57	1.2–2.0
Caprylic acid	.79	0.5–1.0

* The high values are for colostrum milk.

TABLE X

SPECIFIC HEAT OF MILK AND MILK PRODUCTS (AFTER HAMMER AND JOHNSON)

	at 0° C.	at 15° C.	at 40° C.	at 60° C.
Whey	0.978	0.976	0.974	0.972
Skim milk	.940	.943	.952	.963
Whole milk	.920	.938	.930	.918
15% cream	.750	.923	.899	.900
20% cream	.723	.940	.880	.886
30% cream	.673	.983	.852	.860
45% cream	.606	1.016	.787	.793
60% cream	.560	1.053	.721	.737
Butter	.512*	.527*	.556	.580
Butterfat	.445*	.467*	.500	.530

* These values were obtained by extrapolation on the assumption that the specific heat is about the same in the solid as in the liquid state.

INDEX